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VIABILITY AND GERMINATION OF SEEDS AND EARLY LIFE HISTORY OF PRAIRIE PLANTS

By

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VIABILITY AND GERMINATION OF SEEDS AND EARLY LIFE HISTORY OF PRAIRIE PLANTS*

INTRODUCTION

The viability and germination of the seeds and the early development of the seedlings of cultivated crops have received much attention (Duvel, 1904; Weaver, 1926; Weaver and Bruner, 1927; Edwards, 1932). Seed testing has become a part of the routine work of federal and state agricultural departments (Harrington, 1916; U. S. Dept. Agr., 1927). Experiments have been made also on the development of weed seeds (Fawcett, 1908; Oswald, 1908). In connection with problems of reforestation and afforestation, the seeds of trees have been the objects of much research (Hofmann, 1917; Korstian, 1927). Rather recently in range investigations, a few similar studies have been made on the native species of the drier portions of the grassland (Sarvis, 1923; Wilson, 1931).

Practically no work of this nature has been done in the region of tall-grass prairie, centering in Kansas and Nebraska. Native grasslands furnish a very different problem from that of cultivated crops or weeds, since the conditions for germination and establishment are quite different. This research was done at Lincoln, Nebraska. The object was to determine the viability and germination of seeds of prairie plants and the conditions for establishment in the prairie.

The problem was suggested by Dr. J. E. Weaver. The writer wishes to express deep appreciation of the privilege of working under the direction of one who is so thoroughly acquainted with the ecology and physiology of the vegetation of the prairie.

Seeds of the common species were collected each fall during 4 years, from 1928 to 1931. Their viability and germination were tested by planting in soil, usually in the greenhouse. Studies were made of seedlings found in the prairie and of seedlings germinated in sod brought into the greenhouse. Early development was observed from seeds sowed in the greenhouse and in the prairie.

Environment

So closely are the environmental factors of water content of soil, temperature, evaporation, and light related to viability, germination and establishment, that at least brief consideration must be given them. Weaver and Himmel (1931) have recently summarized the factors from measurements made in the field over a period of 12 years.

"Water content and humidity are the master factors of the environment of the prairie. . . . Water content in the surface 6 inches of upland soil varied

* Contributions from the Department of Botany, University of Nebraska, No. 94.

widely and rapidly, often 10 per cent or more during a single week." It approached exhaustion, measured by the hygroscopic coefficient, from one to four times during the growing season. In the surface 3 inches the fluctuations were much more extreme; here the available water was often entirely exhausted. At 6 to 12 inches it was reduced to 2 to 3 per cent once or twice during the season. Below the first foot, the water content was not critical, though the available supply sometimes fell to 5 per cent.

"A close positive correlation was found between precipitation and water content, especially in the surface 6 inches. . . . The mean annual precipitation is 28 inches, of which nearly 80 per cent falls during the growing season." Fourteen inches fell during May, June, and July. For the months important in germination and establishment, the average rainfall of April is under 3 inches, that of May and of June is a little over four. "The rainfall of May and June is usually well distributed but that of July and August is less so. . . . A rainfall of less than 0.20 inch is probably entirely intercepted by the vegetation and the dry surface half-inch of soil", where its effect is extremely temporary. "Drought periods of 15 days or more when the rainfall on consecutive days did not exceed .20 inch occurred every year. . . . That they are rather regularly distributed throughout the growing season is shown by the fact that 6 occurred in April, 9 in May, 5 in June, 9 in July, 7 in August, and 8 in September. . . . The light, scattered showers during such times are of little significance in increasing the water content." The lowering of temperature and increasing of humidity, which are the chief effects of brief showers, are rapidly counteracted by subsequent bright sunshine and wind. Not only is the development of seedlings influenced by such periods of drought but also the process of pollination. In the extreme, long-continued drought of 1930, upland species generally ceased to bloom. The germination of the seeds of the harvest of that year was lower, for many species, than from any of the other years of the study.

"Nebraska has much sunshine; 175 to 185 clear days occur and only 81 to 86 completely cloudy ones. During March, April, and May there is approximately 60 per cent sunshine but June, July, and August have 72 per cent or more."

"Temperatures of air and soil during the long growing season are well within the ranges critical for plants of the prairie and are probably of secondary importance." The average day temperatures of the air were usually between 75° and 85°F. The night temperatures were ten degrees or more lower. The average soil temperature at a depth of three inches was 56° in April, 68° in May, 78° in June, 84° in July, 81° in August and 73° in September. Daily variations of 15° to 18°F. were shown at this depth, but only 1° to 3° at 12 inches. Seeds were thus subjected to considerable fluctuation, a generally stimulating influence for germination (Fivaz, 1931).

"Humidity, through its direct control of transpiration and evaporation from the surface of the soil, frequently determines whether a plant can or can not grow in a given habitat. It must be considered in all problems concerning the distribution of vegetation." During wet years the average day humidity was 50 to 60 per cent. During dry years it was 40 to 50 per cent, falling as low as 15 to 30 sometimes in the late afternoon.

Wind movement, important in the prairie because of the lowering of humidity, is often high. The average hourly velocity is 10.7 miles. "It is fairly constant throughout the year. It reaches a maximum of 13.2 miles per hour in April and decreases gradually to a minimum of 8.7 miles in August." It has frequently less than half these velocities at the six-inch level among the grasses. "Even a movement of 4 to 5 miles per hour greatly increases transpiration. . . . Data show conclusively that the wind is an important environmental factor." The chief effect on establishment is probably through the withdrawal of water from mature vegetation. The small shoots of the seedlings, in the relatively humid atmosphere near the ground, are protected from increases in transpiration. They must, however, have abundant water to continue growth. Indirectly the wind is a powerful influence in depleting the water supply.

"Evaporation varied greatly from year to year. At a height of 2 to 5 inches above the surface of the ground it was usually between 20 and 30 cc. per day, as computed from weekly losses. During periods of drought it sometimes reached 40 to 55 cc. High evaporation was correlated with low humidity and both of these with low water content of soil."

THE PRAIRIE SOD

The prairie is a closed formation. The mass of vegetation is limited by the available water. To within half an inch of the surface, the upper 3 to 6 inches of soil are occupied by roots and rhizomes, and occasionally by bulbs, corms, tubers, and their outgrowths. The dense network of roots extends much deeper. Everywhere the soil is compacted and so thoroughly threaded with plant parts as to form a sod. So well developed is the network of absorbing rootlets that, between absorption and direct evaporation from the surface of the ground, water content of the shallower soil is often reduced below the point available for growth.

More than 95 per cent of the species are perennial. Many of them have a life-span of 10 to 20 years. In competition with these, seedlings find establishment difficult. The large percentage of sunshine, the irregularity of precipitation and the frequent occurrence of high winds after showers are physical factors which combine to keep the surface moisture low. The wetting of the soil around the seed may be too transitory for germination to be effected. Either the seed may fail to swell or, after the embryo has

Fig. 1. Typical upland prairie in late September, after mowing and stacking of hay. (Photo by Weaver).

assumed active growth, it may be destroyed by sudden desiccation. Very frequently the falling seeds are caught and held in the crowns or among the dead stems or leaves of old plants (Fig. 1). If enough water is obtained for germination, the rootlet is in danger of becoming dried beyond recovery before it can elongate into the soil.

The need for water is of first importance. The adjustment to this is rapid penetration. A good root is formed before the shoot appears above ground. A deep absorbing system is produced before much leaf surface is developed (Fig. 2). While these earliest stages of development are progressing in the spring, the established vegetation is adding rapidly to its annual growth of tops. This results in increased competition for water. At the same time there is introduced the factor of reduced light intensity. Practically all prairies are mowed annually. The sod, however, is unbroken. The general effect of removing the foliage cover may not be greatly unlike that produced, under more primitive conditions and more scattered populations, by frequent prairie fires. Shoots start to grow sooner and more vigorously when exposed to the light and warmth of the sun's rays than when they must elongate through a tangle of dead plant parts. According to Weaver and Fitzpatrick (1934) the surface of the soil actually occupied by shoots of grasses and forbs varies from 6.5 to 37 per cent. Sod-forming and bunch-

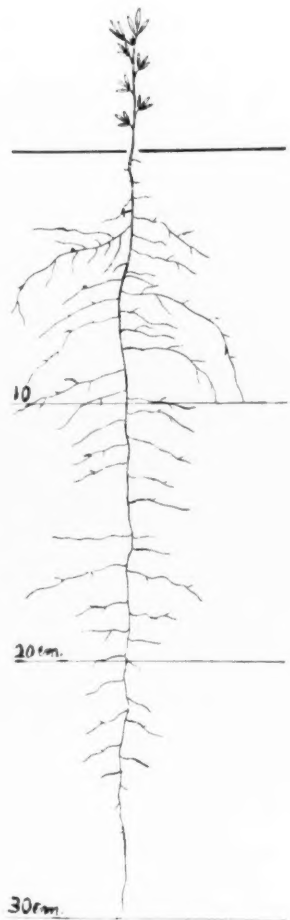


FIG. 2. *Petalostemon candidus* about 6 weeks old, showing rapid penetration of taproot to 30 cm.

forming grasses constitute the bulk of the vegetation. The forbs add an important but variable amount. With an average basal cover of about 15 per cent, the vegetation may have a foliage cover of 100 per cent; that is, the soil may be entirely concealed by the plants when viewed from above. By June the shade under the long established plants has become so dense that seedlings are unable to make adequate carbohydrates for vigorous growth. Before midsummer the light is often reduced to 30 per cent at a height of 12 inches and to 5 per cent at the ground level. The retardation of development usually results in starvation and death (Clements, Weaver, and Hanson, 1929).

VIABILITY OF SEEDS

The absolute viability of seeds can be tested only by very delicate laboratory methods. These include the detection of an electric current, when an induction shock is sent through a living seed which is connected with a

galvanometer, and the observation of an increase in the output of carbon dioxide, when living seeds are electrically stimulated (Tashiro, 1917).

The practical test for viability is germination. A seed must have sufficient vitality to develop a seedling and to maintain it until it is able to support itself. Otherwise there is little significance in the mere demonstration that the embryo possesses enough living protoplasm to make a response to a violent stimulus of an abnormal nature.

General Methods of Testing

The accepted method of seed testing is to expose known numbers of seeds to conditions favorable to germination, usually between layers of moist, absorbent paper or in sand or soil. The numbers of seeds which germinate in a given time are calculated on a percentage basis. The standard of germination is arbitrarily fixed. Most often it is regarded as the projection of both root and plumule beyond the seed coat or as the appearance of the first leaf above ground. Failing to meet such a test, seeds are considered non-viable (Zinn, 1920).

Discussion

For the seeds of ordinary crop plants tested on a commercial scale, such a standard, supplemented by a rigid time limit, has proved satisfactory. One important factor, however, is disregarded. This is dormancy. It may be either primary, due to the condition of the newly formed embryo or seed coat, or secondary, from a relapse into a state which inhibits the resumption of growth.

Cultivation, owing to practical considerations of seeding and harvesting, has resulted in varieties of field and vegetable crops which show much uniformity of behavior. The seeds of most domesticated crops possess very brief latent periods, easily broken under conditions suitable for germination. Native seeds and those of a large number of woody plants classed as "ornamentals" are characterized by long periods of dormancy, frequently recurrent and difficult or impossible to break.

Fawcett (1908) tested germination of weed seeds in Iowa. From plantings at intervals of one month, he concluded that two periods of activity existed, spring and fall, with deep dormancy during the other months.

Howard (1915), working in Missouri for the most part with species other than grasses, found that the larger number of native seeds, planted out-of-doors as soon as they ripened in the summer or fall, did not germinate before the following spring.

Schmidt (1929) observed periodical or seasonal variations in germinative energy. In *Pinus sylvestris*, for example, they were very distinct, with maxima in March-April and minima in late autumn and January.

Darlington (1931), continuing Beal's fifty-year experiment in Michigan,

had occasion to change the time for testing germination from fall to spring. The tests, made at five-year intervals, had been showing successive decreases both in species possessing viable seeds and in numbers of seedlings which germinated. The spring planting yielded a higher record of viability than had been obtained five years earlier.

Wilson (1931), in New Mexico, tested the germination of native grasses for possibilities for range planting. His extensive tables give dates of planting and percentages of germination which suggest the same characteristic.

Death is slower to overtake the seeds of most species when they are stored artificially than when lying in the ground. In addition to the hazards of being consumed by animals or injured by fungi or bacteria, they are exposed to alternate wetting and drying and freezing and thawing. Doubtless many seeds perish because they have begun germination at an unfavorable time. After the seed coats have swollen and the protoplasm of the embryo has been altered to an unstable, more active condition, a sharp decrease in moisture or temperature may desiccate the tissues so rapidly that their essential structure is destroyed.

TESTS OF VIABILITY

Successive plantings of seeds of 10 common prairie species were made at intervals of about 2 months for a year following the collection of seeds in the fall of 1930. Successive plantings of the same species were made at intervals of about one month, following the harvest of 1931, for a period of 8 months. Seven grasses and 3 freely germinating forbs were employed. The list comprised big bluestem (*Andropogon furcatus* Muhl.[1]), little bluestem (*A. scoparius* Michx.), blue grama (*Bouteloua gracilis* (H. B. K.) Lag.), nodding wild rye (*Elymus canadensis* L.), tall panic grass (*Panicum virgatum* L.), Indian grass (*Sorghastrum nutans* (L.) Nash), a dropseed (*Sporobolus asper* (Michx.) Kunth), prairie false boneset (*Kuhnia glutinosa*, Ell. (*K. suaveolens* Fresen.)), a blazing star (*Liatris punctata* Hook.), and common evening primrose (*Oenothera biennis* L.). The hairy grama (*Bouteloua hirsuta* Lag.) and tall marsh grass (*Spartina michauxiana* Hitch.) were included, each for one year.

The plantings were in soil, on benches in the greenhouse. The early plantings were from unfrozen seed. As the seeds were stored at out-of-doors temperatures, a change was made, with the advance of winter, to the use of frozen seeds. Each planting was allowed a period of two months before the count of seedlings was discontinued. Lots of 100 seeds, single or in duplicate, were planted.

The longevity of the seeds of 7 species of grasses and 4 of forbs from one harvest was also tested during four consecutive years. The seeds were kept in dry storage at room temperatures until they were planted. Two

[1] Authority for the name of the species is given only the first time the name is used.

hundred seeds of each species, in duplicate lots of 100 each, were sowed annually in March from 2.5 to 5.5 years after the harvest. The grasses included *Andropogon furcatus*, *A. scoparius*, *Elymus canadensis*, *Koeleria cristata* (L.) Pers., *Sorghastrum nutans*, *Sporobolus asper*, and *Stipa spartea* Trin. *Oenothera biennis*, *Kuhnia glutinosa*, *Liatris punctata*, and *L. scariosa* (L.) Willd. (*L. aspera* (Michx.) Greene) composed the list of forbs.

RESULTS

Power of germination increased gradually from very low or none at harvest time to a maximum in mid-spring. This was followed by a distinct lowering during summer and a marked rise in early fall. If the spring planting was exposed to unfavorable conditions, the germination in early autumn (from seeds of the previous fall) usually was maximum for the year (Tables 1, 2).

Seasonal variability in the time of ripening of seeds was illustrated by a comparison of the earlier plantings of the two years. After the dry, hot summer of 1930, the grasses usually germinated to some extent in October and showed a marked increase in seedling production two months later. The following year there was practically no germination immediately after harvest and very little, in most instances, for several months.

Oenothera biennis in the first year of the study displayed the frequently noted phenomenon of germination at harvest time, followed by complete absence of seedling development for a relatively long period (Crocker, 1916).

Monthly fluctuations of considerable magnitude were the rule with all species. Lack of uniformity of external conditions was undoubtedly responsible to some extent. Such variability is much greater for seeds planted in soil in a general greenhouse than in a thermostatically controlled germinator. The fluctuations, however, are far less extreme than they would be under natural conditions. They should suppress merely the weakest seedlings. These, if developed under uniformly optimum moisture and temperature and without the obstruction of soil to be penetrated, give a false impression of the possibilities of natural germination.

The annual tests of 7 grasses from 2.5 to 5.5 years after they were placed in dry storage at room temperatures displayed, at the end of that period, increased germinative power for 2 species and very marked reduction for 5 species. *Elymus canadensis* decreased in germination from over 95 per cent in its third year to one per cent in its fifth year. Two species of Andropogon and *Stipa spartea* were reduced in their sixth year to about one-tenth of the germination which they gave in their third year. For *Andropogon furcatus* and *A. scoparius* the loss in germinating power developed suddenly, between the fifth and sixth years. *Koeleria cristata* diminished from 21 per cent in the third year to 7 per cent in the sixth. The germination obtained in the pre-

TABLE 1. Percentage germination of seeds of 1930 planted at intervals from October 1930 to September 1931

Species	October 9	December 10	January 10	February 10	April 12*	May 8	June 20	July 18	August 29	September 16
Andropogon furcatus.	1.0	....	4.5	3.0	6.0†	....	6.0†	....	3.5	....
Andropogon scoparius	3.0	8.0†	....	1.5	1.0	....	2.5	5.0	8.0†	....
Bouteloua gracilis...	5.0	31.0†	....	25.5	18.0	....	24.0	....	15.0	....
Bouteloua hirsuta...	0.0	5.0	....	6.0	9.0	....	3.5	....	21.5†	....
Elymus canadensis ..	37.0	....	....	38.0	25.5	....	31.0	....	45.5†	....
Sorghastrum nutans.	4.0	6.0†	....	3.5	0.5	....	0.5	....	4.5	....
Sporobolus asper....	0.0	60.0	....	40.5	....	15.0	....	24.0	....	65.0†
Kuhnia glutinosa....	0.0	45.5	42.0	....	....	57.0†	....	39.0	....	46.0
Liatris punctata.....	3.0	40.0	47.0†	....	....	41.0	....	15.0	....	21.0
Oenothera biennis...	9.0	0.0	....	3.5	....	55.0†	....	28.5	....	....

TABLE 2. Percentage germination of seeds of 1931 planted at intervals of one month from October 1931 to June 1932

Species	October 6	November 10	December 11	January 11	February 15	March 18	April 23	June 3
Andropogon furcatus............	0.0	0.0	7.0	11.0	9.0	5.0	20.0†	3.0
Andropogon scoparius............	0.5	0.0	0.0	1.0	3.0	0.0	5.0†	2.0
Bouteloua gracilis...............	0.0	3.0	1.0	5.0	10.5†	10.0†	10.0†	0.0
Elymus canadensis..............	....	5.0	....	76.0	78.0	....	87.0†	69.0
Panicum virgatum..............	0.5	0.0	0.0	0.0	0.0	0.0	14.0†	1.0
Sorghastrum nutans.............	0.0	0.0	0.0	0.0	1.0	1.0	5.0†	1.0
Spartina michauxiana............	0.0	0.0	0.0	1.0	3.0	....	6.0	7.0†
Sporobolus asper.................	1.0	0.0	0.0	1.0	15.5	32.0	91.0†	18.0
Kuhnia glutinosa.................	6.0	36.0	14.0	49.0	38.0	55.0†	51.0	17.0
Liatris punctata..................	....	7.0	19.0	54.0	67.0	83.0†	70.0	30.0
Oenothera biennis................	0.0	0.0	0.0	0.0	1.5	2.0	13.0	17.0†

*The week following planting in April, 1930 was extremely hot. Low germination of most species was due in part, probably, to drying of the sprouting seeds in the soil.
†Maximum germination.

ceding year was very much lower, emphasizing again the inadvisability of depending on a single test as a basis for conclusions on wild seeds. *Sorghastrum nutans* and *Sporobolus asper* gave their maximum germination in the sixth year. *Sorghastrum nutans* alternated between approximately 15 and 25 per cent throughout the entire experiment. *Sporobolus asper* showed a steady increase from 16 per cent (a low figure for the species) in the third year to 67 per cent in the sixth year (Table 3).

The three composites which were all of one tribe, *Kuhnia glutinosa, Liatris punctata,* and *L. scariosa,* gave no germination in their sixth year.

Large decreases were observed between the fourth and fifth years. *Oenothera biennis* developed a reduction of 50 per cent between the third and sixth years.

The decrease in germinative power was usually accompanied by marked lengthening of the time interval between planting and the appearance of the largest proportion of the seedlings. As the seeds near their limit of life, even in the presence of abundant moisture and suitable temperatures, the chemical reactions which must take place if the protoplasm is to resume an active state, occur slowly or not at all. The generalization frequently made, that species of high and prompt germination are short-lived, was supported by the record of *Kuhnia glutinosa* and contradicted by that of *Sporobolus asper*.

Conclusions

Germination is the practical test of viability of seeds. It fluctuates from month to month for any single lot of seeds of prairie plants. For many species these monthly variations may be large enough to obscure the real viability. A general tendency to yield maximum germination in the spring was seen for native seeds of the prairie region of the central United States. Several species showed a second period of high germinative capacity in early fall, after nearly a year of storage. Immediately after harvest, germination usually was very low. When germination is made the test of viability, it should be tried at the time of year most favorable to the development of the seeds.

Seeds of several common species, kept in dry storage at room temperatures, lost their germinative power within six years. Under the hazards of storage in the surface soil in a prairie climate, their length of life would often be shorter. With the exception of sporadic individuals with unusually hard coats and high vitality, the period of viability in native sod is probably less than five years.

GERMINATION

The germination of seeds of prairie plants was studied in several ways. The most extensive work was in determining the percentage germination of seeds collected in the field when ripe and planted in soil in the greenhouse. Harvests of four consecutive years were tested for 13 grasses and 18 forbs,[2] and for three consecutive years for 2 other grasses and 9 other forbs. In addition several herbs were tested for one or two seasons.

Germination of seeds which had fallen naturally in the prairie was recorded both by list quadrats of seedlings found in the spring and fall during the period of study and also by bringing sod into the greenhouse late in the fall, after most seeds had fallen, and listing the seedlings which developed. These studies of germination in prairie sod were pursued during four consecutive years.

[2] *Amorpha canescens*, a half-shrub under natural conditions, behaves as an herb under mowing. It has been included because of its great abundance and importance.

TABLE 3. Changes in percentage and time of germination of seeds of 1926 planted at intervals of one year*

Species	Germination	1929	1930	1931	1932
Andropogon furcatus	Percentage	13.5	18.5	11.0	1.5
	Time	7	9	12	12–19
Andropogon scoparius	Percentage	8.5	6.0	6.5	1.0
	Time	11	11	31	14
Elymus canadensis	Percentage	96.5	....	1.0	1.0
	Time	10	....	80	33–40
Koeleria cristata	Percentage	21.0	....	0.5	6.6
	Time	10	....	31	12
Sorghastrum nutans	Percentage	14.0	23.5	15.5	26.0
	Time	10	14	12	14
Sporobolus asper	Percentage	15.5	19.5	48.5	67.0
	Time	18	11	14	7
Stipa spartea	Percentage	52.0	....	26.0	5.0
	Time	11	....	13	14
Kuhnia glutinosa	Percentage	26.5	26.5	1.5	0.0
	Time	4	7	25	....
Liatris punctata	Percentage	74.0	52.5	0.0	0.0
	Time	10	8	....	....
Liatris scariosa	Percentage	74.0	81.0	20.0	0.0
	Time	10	11	21	....
Oenothera biennis	Percentage	68.5	....	....	34.0
	Time	8	....	....	19

*Seeds were planted in March. The time of germination is recorded as the number of days when the largest proportion of seedlings appeared.

Problems treated in connection with the percentage germinations of planted seeds include the determination of optimum water content of soil; the effect of freezing, both in dry storage and in moist soil; and the length of the period of dormancy after planting.

TESTS OF GERMINATION

Seeds were collected during their natural period of dispersal in the field and tested for germination during the following winter and spring.

METHODS

The seeds were spread out in the air for a few weeks to promote drying. A small quantity of the seeds of each species was stored in the house and kept at room temperatures until planting. The remainder were stored out-of-doors in cloth sacks protected from precipitation, so that they were subjected to dry freezing from a few weeks to a few months before being planted. Winter temperatures during the 4 years of the study averaged 32°F. for December, 21° for January, 32° for February, and 38° for March. During each of these months fluctuations between average maximum and average minimum temperatures amounted to 15 to 20 degrees. During each winter except the unusually warm season of 1930-1931 temperatures below zero were recorded in 2 consecutive months.

Four hundred seeds of each species were planted. Two hundred of these were from frozen storage, 200 from unfrozen. Both lots were sowed in du-

plicates of 100 each. Occasionally it was necessary to use smaller numbers. Seeds which were conspicuously undersize or poorly filled were avoided when possible.

Examination of the seeds of *Agropyron smithii* Rydb., a species which had given very poor germination, was made, in the fourth year, for the presence of embryos. The seeds were spread in a single layer on a glass plate, illuminated from below by an electric light. By the use of a hand lens it was seen that many of the glumes were empty. These were discarded. This method of improving the chances of germination was very efficacious. It is prevented by the structure of many seeds and is too slow to be practical. It indicates a common cause of low germination.

The seeds were planted in soil on benches in the greenhouse. The soil employed was derived from prairie sod. The appearance of the seedling above ground was the test of germination. After identification the seedling, together with the seed when possible, was removed with little disturbance of the soil. Otherwise damping-off was likely to cause errors in the record.

The first year the seeds were left in the soil for a period of 5 to 6 months. Since most of the germinations occurred during the first 2 months, the length of the test was reduced, after the first year, to 3 months. In the first 3 years the plantings were made in January and February following the harvest. The fourth year all of the sowing was done during the last two weeks of February. Many of the species were also planted at other times during the year. In summer, tests were made in the garden instead of the greenhouse. When more than one test of the previously frozen seeds of a species was made during a year, the one yielding the highest percentage was regarded as expressing the germinative capacity of the species for the year.

CLIMATIC CONDITIONS DURING THE GROWING SEASON

The seeds of the harvest of 1928 produced the highest germination for most of the species tested; those of 1929 the second highest germination. The growing season of 1928 began with low temperatures and low precipitation in April, followed by high temperatures with a continuation of low precipitation in May, before the moisture factor became critical. Low temperatures prevailed in June, so that, while the rainfall was slightly below the average for the month, drought did not occur. Average temperatures in July accompanied by high precipitation prolonged the period of growth. The temperature in August was only slightly above the mean and the rainfall was normal. In September both temperature and precipitation were below normal. During 1929 the mean temperature of April was high, that of May was low. Precipitation was average. June, July, August, and September were in general like the corresponding months of 1928, although the temperatures were slightly higher and the precipitation was somewhat lower.

The seasons of 1930 and 1931, both of which produced poor crops of seeds, were characterized by the termination of growth in midsummer because of severe drought. The excessively high temperatures in June, 1931, with rain only in heavy showers, were apparently more disastrous than the more sustained high temperatures in July, 1930. The temperatures were significant, probably, only because of their bearing on the water relation. In the shallower prairie soils moisture is frequently so near the critical point that while low temperatures may permit growth to continue, high temperatures, by increasing transpiration and evaporation, very soon result in drought. Table 4 summarizes, for the four years, the departures from the mean temperature and precipitation for each of the six months of the growing season.

TABLE 4. Mean monthly temperatures (°F.), mean monthly precipitation (in inches) and departures from the mean during 1928-1931

Year	April		May		June		July		August		September	
	Temperature	Precipitation	Temperature	Precipitation	Temperature	Precipitation	Temperature	Precipitation	Temperature	Precipitation	Temperature	Precipitation
Average of 50 years	52	2.5	62	4.0	71	4.2	77	3.9	75	3.5	70	2.9
1928	− 4	−1.3	+ 3	−1.3	− 5	−0.3	0	+1.9	+ 1	+0.1	− 2	−0.9
1929	+ 2	+0.4	− 2	−0.8	− 1	−2.4	+ 1	+1.5	+ 2	−2.6	− 2	−1.1
1930	+ 4	+0.2	0	−0.9	0	−1.3	+ 6	−2.8	+ 2	+0.2	+ 1	−1.6
1931	+ 1	−0.8	− 3	+2.4	+ 7	+0.2	+ 3	−1.8	0	+0.3	+ 8	+0.9

DISCUSSION

It becomes apparent that the germination often varied considerably for one lot of seeds at different times of year. A species might pass out of the condition of initial dormancy which regularly follows harvest and give a large yield of seedlings from the winter planting. The following year the same species might show only low germination from the winter sowing while subsequently it produced a higher percentage, comparable to that of the preceding year; *e.g. Sporobolus asper* and *Oenothera biennis*. Further plantings in the same year might again show a reduced percentage. This might be due to return of dormancy, or, less often, to some unfavorable external condition, such as brief periods of excessive heat during tests carried on in the spring.

The majority of species were tested only once during a year, by plantings made in January and February. Work done in the course of the study indicated that it would have been better in general to have delayed sowing until 3 months later. Continuing the tests started in late winter through the spring months is not the equivalent of starting them in the spring. General correlation exists, as emphasized in commercial seed testing, between promptness

of germination and number of seedlings. This correlation is less marked in wild seeds.

Dormancy was still apparent in late winter when most of the tests were made. Species and individuals in a group of seeds of one species pass out of dormancy at different times. Such a species as *Aristida oligantha* Michx., giving a consistent, fairly high germination (30 to 40 per cent) in each planting of the three winters in which it was tested, might reasonably be assumed to have thrown off the restraints to germination. *Sporobolus asper*, on the other hand, varied in its response from year to year. The seeds of the harvests of 1928 and 1929 were mostly dormant during the winter tests. The highest germination was 7 per cent, though nearly 60 per cent was obtained a few months later. The harvest of 1930 gave 40 per cent from the February planting and a maximum of 57 in the following autumn. The forb *Salvia pitcheri* Torr. produced from the harvest of 1929, during the last half of January, a germination of 50 per cent. The next harvest, planted early in February, yielded only 15 per cent. Placed in the soil May 8 and left for three months, seeds of the same crop gave 40 per cent germination within two weeks, followed by almost complete cessation. Resumption of seedling formation during the last week of July raised the total to 61 per cent.

Relatively few of the forbs were tested except at the winter planting. In general they gave extremely poor results when sowed in summer for special tests. In several instances better germination was obtained from plantings made in the greenhouse in the spring than from winter tests. The influence of time of planting appeared to vary with species. Possibly this phase of behavior in germination, or more accurately in dormancy, is characteristic of a genus. Howard (1915) working with a large number of native species in Missouri, observed that the species of a family appeared to "show more or less the same characteristics as regards time and percentage of germination".

The general results expressed below are stated with the realization that it may not be the true germinative capacity of the seeds which is apparent, but germination restricted by dormancy.

RESULTS

Most grasses gave less than 10 per cent germination in at least one or two of the 4 years. *Agropyron smithii* remained in this group during 3 years, with percentages lying between 0.5 and 5.5. The fourth year specially selected seeds, known to contain embryos, gave 48 per cent.

Andropogon furcatus, *Bouteloua hirsuta*, and *Panicum virgatum* gave between 10 and 20 per cent during two of the 4 years. Their germination during the other years was lower. *Andropogon scoparius* during one year only showed a germination which was far above 10 per cent. The subclimax *Aristida oligantha* and the two weeds, *Chaetochloa glauca* Scribn. and *Pani-*

cum capillare, during both years in which they were tested, gave 20 to 40 per cent. *Bouteloua gracilis* and *Bulbilis dactyloides* (Nutt.) Raf. both produced germinations between 20 and 40 per cent during one year of the 4, between 10 and 20 during another year, and below 10 per cent during the first two years. *Elymus canadensis* always gave over 40 per cent germination and during two years over 60 per cent. The record of *Koeleria cristata* was above 40 per cent one year, between 20 and 40 during one year, and between 10 and 20 per cent during two years.

Inspection of the data (Tables 5 to 7) with the fact in mind that the winter plantings, especially during the first two years, were somewhat premature, leads to the following generalizations. A germination of 40 to 60 per cent may be expected from *Elymus canadensis*, *Poe pratensis* L., and *Sporobolus asper*. Between 20 and 40 per cent may be predicted for *Aristida oligantha*, *Chaetochloa glauca*, and *Panicum capillare*. Between 20 and 40 per cent should be induced in *Bouteloua gracilis* and *Koeleria cristata*. Koeleria is capable of giving a higher yield. Ten to 20 per cent should be obtained from *Andropogon furcatus*, *Bouteloua hirsuta* and, at least in some years, from *Andropogon scoparius*, *Bouteloua curtipendula* (Michx.) Torr., and *Panicum virgatum*. While less than 10 per cent germination was obtained from *Sorghastrum nutans*, *Spartina michauxiana*, and *Stipa spartea* during the four years of 1928 to 1931, these species are capable of giving better germination under proper conditions of seed formation, ripening and storage. Seeds of *Sorghastrum nutans* gathered in

TABLE 5. Percentage of germination of seeds of grasses of four harvests in successive tests. Percentages from unfrozen seeds are given in parentheses

Year of harvest	Year of test	Season of test	Andropogon furcatus	Andropogon scoparius	Bouteloua curtipendula	Bouteloua gracilis	Bouteloua hirsuta	Elymus canadensis	Koeleria cristata	Panicum virgatum	Sorghastrum nutans	Sporobolus asper	Spartina michauxiana
1928	1929	Winter	(2.0)	(27.5)	....	(2.5)	(11.0)	(84.0)	....	(1.0)	(5.5)	(16.5)	....
		Winter	2.5	37.0	5.5	3.0	7.0	81.0	26.0	2.5	8.5	6.5	....
	1930	Winter	5.0	11.0	....	....	2.0	....	....	....	4.0	....	....
	1931	Winter	.3	....	....	....	....	....	....	....	....	....	....
1929	1930	Winter	(.5)	(2.5)	(11.5)	(2.5)	(0.0)	(61.0)	(49.5)	(1.0)	(0.0)	(5.0)	(8.0)
		Winter	5.0	8.5	10.5	7.5	3.5	50.5	....	2.5	3.0	3.0	10.5
		Spring	3.0	....	....	....	10.5	....	54.0	....	....	....	....
		Summer	19.0	....	6.0	....	....	23.0	....	....	4.0	36.0	....
		Fall	3.0	....	....	0.0	1.5	....	....	3.0	2.0	59.0	....
1930	1930	Fall	(1.0)	(8.0)	....	(31.0)	(5.0)	(37.0)	....	(3.0)	(6.0)	(60.0)	(0.0)
	1931	Winter	(1.0)	(2.0)	(0.0)	(22.0)	(10.5)	(32.0)	(29.0)	(0.0)	(1.0)	(77.0)	(3.0)
		Winter	4.5	.5	0.0	25.5	6.0	38.0	10.0	0.0	3.5	40.5	0.0
		Spring	6.0	1.5	2.5	18.0	9.0	25.5	1.5	1.0	.5	15.0	1.5
		Summer	6.0	5.0	.5	24.0	3.5	31.0	10.0	1.5	.5	24.0	....
		Fall	3.5	8.0	3.5	15.0	21.5	45.5	....	11.5	4.5	57.5	1.0
1931	1931	Fall	(7.0)	(0.0)	(1.0)	(3.0)	(1.0)	(18.0)	(4.0)	(0.0)	(0.0)	(0.0)	(0.0)
	1932	Winter	(.5)	(4.0)	(0.0)	(6.5)	(8.5)	....	(18.0)	(1.5)	(2.0)	(3.0)	(1.0)
		Winter	9.0	3.0	0.0	10.5	5.5	77.9	63.0	0.0	1.0	15.5	3.0
		Spring	20.0	5.0	....	10.0	....	87.0	....	14.0	5.0	91.0	6.0
		Summer	3.0	2.0	....	....	....	69.0	9.0	1.0	1.0	18.0	7.0

TABLE 6. Percentage of germination of seeds of forbs of four harvests in successive tests

Year of harvest	Year of test	Season of test	Amorpha canescens	Helianthus rigidus	Kuhnia glutinosa	Liatris punctata	Liatris scariosa	Oenothera biennis	Salvia pitcheri	Silphium integrifolium
1928.......	1928	Fall.....	(0.0)	(2.0)	(26.0)	(49.0)	(22.5)	(0.0)	....	(5.5)
	1929	Winter...	(6.0)	(10.5)	(54.5)	(39.5)	(54.5)	(36.5)	....	(28.0)
		Winter...	21.5	11.0	53.0	69.5	61.0	45.5	....	28.0
	1930	Winter...	....	....	65.0	45.5	45.0	....	....	....
1929.......	1930	Winter...	(3.0)	(11.5)	(39.0)	(51.5)	(55.5)	(55.5)	(49.0)	(12.0)
		Winter...	27.5	4.0	49.0	42.5	64.0	48.0	56.5	11.0
		Summer..	24.5	....	....	....	....	....	24.0	....
1930.......	1930	Fall.....	....	....	(45.5)	(40.0)	....	(9.0)	....	....
	1931	Winter...	(1.0)	(0.0)	(54.0)	(59.5)	(1.8)	(2.5)	(47.0)	(45.0)
		Winter...	5.0	3.5	47.0	47.0	34.5	3.5	15.0	51.5
		Spring...	8.5	2.5	57.0	41.5	25.0	58.5	61.5	47.0
		Summer..	12.0	.6	39.0	15.0	....	45.5	3.0	9.0
		Fall.....	....	....	46.0	21.0	....	....	....	....
1931.......	1931	Fall.....	....	....	(39.0)	(14.0)	(0.0)	(0.0)	....	(1.0)
	1932	Winter...	(3.5)	(1.0)	(34.0)	(62.5)	(17.0)	(2.5)	....	(2.0)
		Winter...	31.0	0.0	49.0	67.0	45.0	1.5	....	.5
		Spring...	....	....	55.0	83.0	....	13.0	....	....
		Summer..	....	0.5	17.0	30.0	6.0	17.0	....	....

1926 and stored for two years before any tests were made consistently gave germinations lying between 15 and 25 per cent during four consecutive years. *Stipa spartea*, from the harvest of 1926, tested in its third year, gave 50 per cent. The germination from *Spartina michauxiana* was always small. With the exception of two plantings it gave less than 5 per cent.

The forbs as a whole showed higher germination from the harvests of 1928 and 1929 than from those of 1930 and 1931. This was the more conspicuous because practically the only tests made during the first two years were those of the regular winter plantings. The percentage from the two later harvests were sometimes increased by additional tests in the spring. The highest figure obtained from all tests of dried and frozen seeds during a year was regarded as expressing the germination for a given harvest.

Species giving over 40 per cent germination at least two years, and usually three out of four seasons, included *Anemone cylindrica* A. Gray, *Kuhnia glutinosa, Liatris punctata, L. scariosa, Oenothera biennis, Physalis lanceolata* Michx., *Tragopogon pratensis* L., and *Salvia pitcheri*. Both Anemone and the ruderal Tragopogon produced germinations of more than 90 per cent from one of the harvests. Physalis gave a maximum of 83 per cent. Kuhnia showed a maximum of 65, with a frequent germination of 50 per cent. *Liatris punctata* produced a maximum of 83 per cent, though its germination was usually between 50 and 70 per cent. *Liatris scariosa* showed the same range. The maximum, 81 per cent, was obtained from a harvest previ-

TABLE 7. Percentages of germination of seeds of four harvests, tested once only. Percentages from unfrozen seeds are given in parentheses

Year of harvest	1928	1929	1930	1931	Year of harvest	1928	1929	1930	1931
Year of test	1929	1930	1931	1932	Year of test	1929	1930	1931	1932
Agropyron smithii	(11.5)	(1.0)	(0.0)	(6.0)	Hieracium longipilum	....	....	(10.0)	(53.0)
	5.5	2.5	.5	49.0		2.8	....	24.5	18.5
Aristida oligantha	(23.0)	(44.0)	(25.5)	....	Lespedeza capitata	(1.0)	(2.5)	(0.0)	(0.0)
	39.5	42.0	30.5	....		3.5	4.0	0.0	0.0
Bulbilis dactyloides	(8.0)	(10.5)	(14.0)	(2.5)	Meibomia illinoensis	(8.5)	(3.5)	(13.0)	(3.5)
	7.0	14.5	33.0	5.5		46.0	7.0	21.0	17.5
Chaetochloa glauca	(37.5)	(7.5)	....	....	Petalostemon spp.	....	(2.5)	....	(.5)
	38.0	22.5	....	....		....	12.5	9.0	7.0
Panicum capillare	(27.0)	(5.0)	....	....	Physalis lanceolata	(76.5)	(59.5)	(47.0)	(14.5)
	23.5	40.5	....	....		83.5	67.0	48.0	19.5
Stipa spartea	....	(4.3)	(3.2)	(2.5)	Rosa arkansana	(.5)	(0.0)	(0.0)	(0.0)
	....	8.0	3.0	0.0		0.0	0.0	0.0	0.0
Carex festucacea	....	(.5)	(0.0)	....	Silene antirrhina	....	....	....	....
	....	.5	.5	.5		....	69.3	47.0	....
Achillea occidentalis	....	(2.7)	(29.0)	(18.5)	Silphium laciniatum	....	....	(31.0)	....
	....	7.5	36.0	24.0		....	0.0	45.0	....
Anemone cylindrica	(70.5)	...	(15.5)	....	Silphium perfoliatum	....	....	....	....
	91.0	49.5	10.0	....		....	8.8	50.0	....
Antennaria campestris	....	(4.5)	(8.0)	(4.0)	Sisyrinchium angustifolium	....	(0.0)	....	(0.0)
	....	0.5	3.0	3.5		....	....	2.0	0.0
Artemisia gnaphalodes	(12.5)	(0.0)	(0.0)	(0.0)	Solidago altissima	(1.5)	....	(0.0)	(1.0)
	21.0	0.0	0.0	0.0		3.0	12.0	1.3	0.0
Aster multiflorus	(1.0)	(.5)	(0.0)	(5.5)	Solidago glaberrima	....	(6.0)	....	(0.0)
	9.0	7.0	.5	.5		....	12.0	....	.5
Aster salicifolius	(8.0)	(3.0)	(0.0)	(10.0)	Solidago rigida	(4.0)	(16.5)	(0.0)	....
	9.5	3.0	1.0	5.5		6.0	15.0	.5	....
Astragalus canadensis	(.5)	(5.0)	....	....	Tragopogon pratensis	....	(74.0)	(71.0)	(58.5)
	2.5	2.0	3.5	....		....	95.0	70.5	54.0
Echinacea pallida	(3.5)	(3.5)	(3.5)	(1.0)	Verbena stricta	(2.0)	(3.0)	(.5)	(0.0)
	1.5	7.0	5.0	1.5		3.0	6.5	.5	0.0
Glycyrrhiza lepidota	(0.0)	(1.0)	(1.0)	....	Vernonia baldwini	(26.0)	(30.0)	(2.5)	(0.0)
	1.0	2.0	.5	....		15.0	11.0	11.5	1.0
Grindelia squarrosa	(35.5)	(11.5)	(9.5)	....					
	50.0	9.5	17.0	....					

ous to the collections of this study, tested after 3.5 years of dry storage. *Oenothera biennis* gave a maximum of 58 per cent. Usually the germination was 45 to 50 per cent. *Amorpha canescens* Pursh gave about 30 per cent in the tests of two years, somewhat over 20 per cent one year and 12 per cent after the hot summer of 1930. *Meibomia illinoensis* (A. Gray) Kuntze produced 46 per cent during one year, about 20 per cent in the tests of two other years and less than 10 per cent from its poorest harvest. *Grindelia squarrosa* (Pursh) Dunal varied from 10 to 50 per cent during three years of testing. Silphium spp. produced 45 to 50 per cent as maximum germination. The yields from the rosin weeds were small, owing to irregularity of germination, unless they were left in the soil for a period of six months or more. *Achillea occidentalis* Raf. gave percentages of 36 and 24 in two of the three tests. The third harvest, collected very late in the season, gave less than 10 per cent.

Artemisia gnaphalodes Nutt. produced 21 per cent of seedlings from the harvest of 1928. The three later crops failed to germinate. Few seeds could be found in the dried flower heads. *Vernonia baldwini* Torr. showed germinations of 10 to 15 per cent during three years out of four. Three species of goldenrod, *Solidago altissima* L., *S. rigida* L., and *S. glaberrima* Martens (tested two years only) gave germinations of 12 to 15 per cent from the harvests of 1929. Usually the percentage was less than five. *Helianthus rigidus* (Cass.) Desf. produced 11 per cent from the seeds of 1928 and less than 5 per cent during other years. Two species of Petalostemon gave about 10 per cent in several tests. Less than 10 per cent was obtained in any season from *Aster multiflorus* Ait. (*A. ericoides* L.), *A. salicifolius* Lam., *Echinacea pallida* (Nutt.) Brit., and *Verbena stricta* Vent.; less than 5 per cent from *Antennaria campestris* Rydb. and *Astragalus canadensis* L. (*A. carolinianus* L.). *Lespedeza capitata* Michx. gave less than 5 per cent, except from a test of seeds gathered two years previous to the beginning of this experiment. These, tested after two and one-half years of storage, yielded 14 per cent. *Glycyrrhiza lepidota* Nutt., *Rosa arkansana* S. Wats. (*R. suffulta* Greene), and *Sisyrinchium angustifolium* Mill. (tested two years) did not exceed two per cent (Tables 6 and 7).

CONCLUSIONS

Most prairie species produce large numbers of seeds. Small but by no means negligible percentages of these are viable. The number of seedlings obtained varies greatly with the growing season preceding the harvest. Species producing seeds which give germinations of 50 to 80 per cent when grown in relatively cool, moist summers, are greatly handicapped in seed formation during the more frequent seasons characterized by periods of drought. The same species, particularly if drought occurs before midsummer, may produce seeds which give a germination of less than 20 or even less than 10 per cent.

The time of year when a test is made is an important factor for seeds in which it is hard to break dormancy. A given lot of seeds which produces extremely low germination or none from a single test may yield much better results at a more favorable time. The spring, particularly April and May, has been found to be the season when native prairie seeds produce seedlings most readily and abundantly. Early fall of the year subsequent to that of the harvest proved to be second only to spring, and occasionally more favorable, as a season for germination. Once the seeds are thoroughly ripened, some species seem to be almost as dependable as cultivated crops; *e.g. Bouteloua gracilis, Sporobolus asper, Amorpha canescens,* and *Salvia pitcheri.*

The seeds of most prairie plants are subject to deep dormancy during the greater part of the year. Wild seeds, however, are far less certain than those of cultivated species to be killed by subjection to conditions conducive to

germination at times unfavorable for development. They often remain latent, no doubt with somewhat lowered vitality. While many die, a surprisingly large number will develop if conditions again become suitable during the season which is normal to them for germination.

RELATIVE GERMINATION OF FROZEN AND UNFROZEN SEEDS

Two hundred frozen and 200 unfrozen seeds of each species were planted in the winter tests of germination. The duplicate lots of 100 seeds each were sowed at the same time.

RESULTS

Slight but probably definite superiority in germination was observed, during tests of 3 or 4 consecutive years, from frozen seeds. Inconsistencies were common from season to season, so that the results from a single year would not have been a safe criterion of the influence of freezing. In comparisons of the germinations following the 2 treatments of stored seeds (Tables 5 to 7) differences of more than 5 per cent have been regarded as large; 5 to 2 per cent as small, and under 2 per cent as insignificant.

Andropogon furcatus and *Bouteloua gracilis* alone, of 13 grasses tested through 4 consecutive years, always produced higher germination from frozen than from unfrozen seeds. The differences were never large. Five species gave higher germination from frozen than from unfrozen seeds during 3 years out of 4 or 2 years out of 3: *Agropyron smithii, Aristida oligantha, Panicum virgatum, Sorghastrum nutans,* and *Spartina michauxiana.* The excess of seedlings from unfrozen seeds, which occurred in one year only, was small except for *Agropyron smithii. Andropogon scoparius* produced distinctly higher germination from frozen seeds of 1928 and 1929 and slightly lower germination from frozen seeds of 1930 and 1931.

Frozen seeds of several forbs always produced more seedlings than unfrozen seeds. Such species were *Achillea occidentalis, Amorpha canescens, Lespedeza capitata, Liatris scariosa,* species of Petalostemon and *Physalis lanceolata.* The advantages of frozen seeds were always large for these species with the exception of *Lespedeza capitata. Antennaria campestris* only among the forbs consistently gave higher germination from unfrozen seeds. The germination of this species was always low. When forbs produced a higher yield from unfrozen seeds, the difference was small or insignificant in twice as many instances (20 specific cases) as it was large.

DISCUSSION

While only the winter sowing was made to test the unfrozen crops, the frozen seeds were planted, in many species, two to several times during one or more years. The percentage differences shown by one species in successive tests during a year often exceeded the differences between frozen and

unfrozen seeds. Many seeds at least during some years, had not completed ripening by late winter or were still in a condition of deep dormancy. *Oenothera biennis* furnished a clear example of this. Seeds of 1928, planted January 31, 1929, gave little germination until March. Between the middle of March and the last of May they gave a total germination of 45.5 per cent from the frozen seeds and 36.5 from the unfrozen. The seeds of 1929, planted January 14, began to germinate late in February. By the middle of April the frozen seeds had produced 48 per cent germination and the unfrozen 55.5. By fall they had returned to the dormant condition, as at that time they gave only 3 per cent.

The unfrozen seeds of Oenothera varied in general with the annual fluctuations of the frozen seeds planted at the same time. The first two years the yields of both were high; the last two years the winter yields were very low.

In a few instances freezing seemed to have a distinctly injurious effect. Seeds of *Hieracium longipilum* Torr. from the harvest of 1931 when unfrozen yielded 53 per cent germination; after freezing, 18.5. The preceding year, on the other hand, the unfrozen percentage was 10 and the frozen exceeded 20. Among grasses, *Elymus canadensis* and *Sporobolus asper* sometimes showed, when sown in winter, distinctly better germination from unfrozen than from frozen seeds. No external irregularity in conditions could be observed to account for such inconsistencies.

CONCLUSIONS

Freezing of seeds in dry storage probably tends to accelerate the processes which cause a seed to break dormancy and respond to conditions favorable to growth (Pack, 1921). This is indicated by a somewhat higher percentage of germination from seeds which have been exposed, in a dry condition, to winter temperatures of 20 to 32°F. for several weeks, compared with seeds which have been kept for the same length of time at room temperatures (60°F. and usually above). For a few species distinct improvement could be seen. The increase, however, was seldom large enough or consistent enough to be of unquestionable significance.

LENGTH OF PERIOD OF DORMANCY AFTER PLANTING

The interval between the planting of seeds and the appearance of seedlings above ground shows much variation in any native species. The latent periods have been computed mainly from the winter tests, though comparisons have been made with the incubation time shown by plantings at other seasons. The number of days between the date of sowing and the approximate date on which the largest proportion of new seedlings appeared has been considered more important than the time of the first germination. The time of the last germination is probably relatively insignificant. Almost any lot of

wild seeds contains a few members which resist development for a long while and finally germinate if left in moist ground for many weeks.

The latent period varies with the condition of the seed. Within a species, an abnormally long interval before germination usually accompanies the production of a low percentage of seedlings. The period of dormancy in the soil is generally shorter in the last half of the spring than in winter or in midsummer.

RESULTS

A definite latent period was found for the seeds of only a few species. The largest number of seedlings of a given species usually appeared in practically the same number of days from both frozen and unfrozen seeds. In one or occasionally 2 years out of 3 or 4, a few grasses and a considerably larger number of forbs showed a much briefer period of dormancy for frozen than for unfrozen seeds. Less than a third as many instances were recorded of briefer dormancy of unfrozen seeds. In most of these instances, the larger number of seedlings was produced from the type of storage characterized by the shorter latent period.

The seeds studied have been grouped into four classes of rather wide range in respect to incubation period in the soil. Exceptions to all of these groups occur. The time applies to plantings made at fairly appropriate seasons of the year. Favorable conditions, either external or internal, may greatly shorten the dormancy for a few seeds of any species.

Species germinating in less than two weeks include the greater number of the grasses tested: *Andropogon furcatus*, Bouteloua spp., *Bulbilis dactyloides, Chaetochloa glauca, Elymus canadensis, Koeleria cristata, Panicum capillare, Poa pratensis,* and *Sporobolus asper.* Most of the forbs were about equally divided between the group which germinated within 2 weeks and the group which required between 2 and 4 weeks. Those germinating in less than 2 weeks included *Achillea occidentalis, Amorpha canescens, Astragalus canadensis, Grindelia squarrosa, Hieracium longipilum, Kuhnia glutinosa, Liatris punctata, Oenothera biennis, Salvia pitcheri,* and *Tragopogon pratensis.* Most of the species of this group were capable of giving good germination within ten days.

Species requiring from 2 to 4 weeks for germination included *Agropyron smithii, Andropogon scoparius, Aristida oligantha, Panicum virgatum,* and *Sorghastrum nutans,* and a much larger number of forbs. The latter were represented by *Antennaria campestris, Aster salicifolius, Echinacea pallida, Glycyrrhiza lepidota, Helianthus rigidus, Lespedeza capitata, Meibomia illinoensis,* Petalostemon spp., *Physalis lanceolata,* Salidago spp., and *Vernonia baldwini.*

Species producing most of their germinations between 4 and 6 weeks after sowing included the two grasses *Spartina michauxiana* and *Stipa spartea*

and the forbs *Anemone cylindrica*, *Aster multiflorus*, and *Verbena stricta*. *Liatris scariosa* usually occurred in this group in the winter plantings. It always required considerably more time than *L. punctata*.

Species requiring more than six weeks in the soil before germination became abundant included 3 species of Silphium. *Artemisia gnaphalodes*, in the one year in which it germinated, appeared in the same group. The rosinweeds were characterized by irregularity in time of germination. At the end of the usual 3 month tests they were beginning to develop somewhat freely. The seeds of the harvest of 1930 were left in the ground for a period of 8 months, from late winter to mid-fall. Under careful watering seedlings appeared, a few at a time, throughout the summer and the first half of September. Germination of 3 species of Silphium was increased from 10 to 18 per cent at the end of 3 months to totals of 45 to 50 per cent. No definite period could be marked off as the time during which the largest number of germinations occurred.

CONCLUSIONS

The length of the latent period for seeds planted in soil varies greatly with the season of planting. If this is favorable, most grass seeds will germinate within 2 weeks. Most of the forbs belong to one of two groups of about equal numbers: those which germinate in less than 2 weeks and those which require 2 to 4 weeks. A few species of both grasses and forbs seldom produce many seedlings until the second month after planting.

Germination of Seeds Frozen in Moist Soil

The effect of freezing seeds planted in moist soil was tested for 15 grasses, one sedge and 26 forbs, all from the harvest of 1931. The experiment was started in the early winter of 1931 and continued through the following spring. Two years later the experiment was supplemented by a repetition with 7 species of grasses and 10 of forbs from the harvest of 1933.

METHODS

The seeds were dried in the air and stored indoors until December. They were then planted in duplicate lots of 100 each. Plantings were made in large flats filled with screened prairie soil of good water content. The seeds were covered with a layer of earth of a thickness equal to about 4 times their diameter. This was pressed firmly over them and well watered, to insure contact of the seeds with a supply of moisture. The flats were covered with cheesecloth to prevent entrance of foreign seeds and placed out-of-doors until the end of the experiment. Records of germination were kept until June.

RESULTS

Nearly all of the germination occurred during April. At the first observation, April 5, many very young plants were present. In the majority of

the species they formed relatively large, even stands. This was conspicuous for several species characterized in the greenhouse by very uneven development. Examples were rosin-weed with 50 seedlings in one of the duplicate lots and 68 in the other; two species of goldenrod with 15 to 20 in each lot; and tall marsh grass with 4 in one duplicate and 11 in the other. Very little germination occurred after May 5. Amorpha and Meibomia constituted exceptions, with about half of the seedlings of each appearing between May 5 and 19.

Higher germination under stratification than from either frozen or unfrozen dry storage was given by 7 of the 14 species of grasses. The percentages were 2 or 3 times as large as maxima for the year obtained from dry storage. The percentage germinations from stratification and from seeds stored dry were respectively: *Andropogon scoparius,* 11 and 5; *Bouteloua curtipendula,* 5 and 0; *B. hirsuta,* 21 and 8; *Bulbilis dactyloides,* 35 and 6; *Koeleria cristata,* 58 and 18; *Spartina michauxiana,* 12 and 7; *Stipa spartea,* 9 and 3 per cent. Fewer seedlings were produced from stratification than from dry storage by *Andropogon furcatus* (13.5 as against 20 per cent), *Bouteloua gracilis* (6.5 and 10.5), *Elymus canadensis* (55.5 and 87), *Poa pratensis* (57.5 and 78.5) and *Sporobolus asper* (83 and 91). *Panicum virgatum* (14 per cent), and *Sorghastrum nutans* (5 per cent) gave maximum germination from dry storage equal to that from stratification. *Carex pennsylvanica* Lam. increased in germination from 0.5 for seeds frozen dry to 3 per cent for those which were layered.

In the supplementary experiment 6 of the 7 species of grass gave distinctly higher germination under stratification than without it. The seventh yielded practically the same by both methods. The three which agreed definitely with the results of the original experiment were *Bouteloua hirsuta* (11 per cent stratified; 8, not stratified), *Bulbilis dactyloides* (42, stratified; 3, unstratified), and *Spartina michauxiana* (46, stratified; 21, not stratified). *Panicum virgatum* and *Sorghastrum nutans,* which had showed no improvement in the previous experiment, increased markedly under stratification, the panic grass from 2 to 8 per cent and the Sorghastrum from 45 to 60. *Andropogon furcatus,* contrary to the effect obtained earlier, produced twice as much germination under stratification (15 per cent, compared to 7 per cent without it). *Andropogon scoparius,* which had formerly improved in yield under stratification, gave 18 per cent stratified and 19 per cent after dry freezing.

Twenty of the 26 species of forbs in the first test gave distinctly higher germination from the seeds which lay in moist soil out-of-doors all winter than from those which were stored dry. The percentage from stratified seeds was usually 2 or 3 times higher and often much more. *Achillea occidentalis,* for example, increased from 27 to 63 per cent; *Antennaria campestris* from 4 to 9; *Aster multiflorus* from 5 to 10; *A. salicifolius* from 10 to 28;

Echinacea pallida from 2 to 38; *Helianthus rigidus* from 1 to 19.5. Two species increased slightly: *Erigeron ramosus* (Walt.) B. S. P. from 10 to 12 and *Rosa arkansana* from 0 to 0.5 (differences too small to be significant). *Lespedeza capitata* increased from 0 to 4 per cent; *Petalostemon candidus* (Willd.) Michx. from 9 to 17; *Physalis lanceolata* from 19 to 73; *Psoralea floribunda* Nutt. from 9 to 24; *Silphium integrifolium* Michx. from 2 per cent, at the end of the 3-month incubation period of the winter test, to 65 per cent; *Solidago altissima* from 1 to 22.5 per cent; *S. glaberrima* from 0.5 to 37; *Vernonia baldwini* from 1 to 44. Three species which gave no germination from either type of dry storage produced good or high percentages from stratification: *Pentstemon grandiflorus* Nutt., 35 per cent; *Sisyrinchium angustifolium*, 17; *Verbena stricta*, 53. All 3 species in previous years had given very little germination or none.

Four forbs produced lower germination under stratification than from either kind of dry storage. *Hieracium longipilum* was reduced from 53 per cent (unfrozen) and 18 (dry frozen) to 0 per cent when layered. *Kuhnia glutinosa* was reduced from 55 per cent from dry frozen storage to 22 when layered, and *Liatris punctata* from 83 to 26. *Tragopogon pratensis* showed slight differences in germination: 58 per cent from unfrozen seeds, 54 from those which were frozen dry, 53 from stratification. *Liatris scariosa*, with a maximum germination of 45 per cent from dry frozen seeds, decreased under stratification to 29 per cent. *Amorpha canescens* also decreased in yield, from 31 per cent after dry freezing to 17 from stratified seeds.

The supplementary stratification of 10 forbs two years later greatly increased the germination of 6 species and produced little or no apparent change in the seeds of 4 species. No significant decreases were observed. The same degree of improvement noted the first year was obtained in the second test of *Physalis lanceolata* (69 per cent with stratification and 12 per cent without it), *Verbena stricta* (20 per cent with it and 7 without) and *Vernonia baldwini* (42 and one per cent respectively). *Solidago canadensis* increased to 11.5 from 0 under the treatment. *S. altissima*, however, which had increased from one to 23 per cent in the first stratification experiment, yielded 29 per cent from dry freezing and dropped slightly to 27 per cent when stratified. *Aster multiflorus*, which had increased from 5 per cent to 10 in the first stratification, made an insignificant rise in negligible germination from 0 to 0.5. *Amorpha canescens* also gave too small germination by both methods (between one and 2 per cent) to be considered. *Helianthus rigidus*, which had risen from one to 19.5 when stratified after the previous harvest tested, gave equal yields of 17 per cent. Two closely related composites which had appeared to be seriously injured by freezing in the ground underwent as great a change, in the supplementary experiment with a subsequent harvest, in the opposite direction; *Kuhnia glutinosa* (60 per cent,

stratified and 25, non-stratified) and *Liatris scariosa* (63 stratified, and 36, not stratified). No other forbs were tested.

DISCUSSION

In considering the effect of stratification, the first problem is to determine the germinative capacity of a species under dry storage. The highest percentage obtained at any time during a year has been regarded as the germinative capacity for that year. Owing to fluctuations shown by most species in the course of a year, it is impossible to be certain of the potential germination from the numbers of seedlings observed. The maximum from the entire four years of the study probably constitutes the best basis obtainable from these data for estimating the germinative power of a species. Comparison of the results of stratification have been made, accordingly, with the two highest records of each species during the entire period of work.

The harvest of 1931 from which stratified seeds were taken was in general poor and resulted in low germination from dry stored seeds of many species. The percentages from stratified seeds may have been lower also than if they had been from another harvest. There is some evidence, however, that stratification tends to improve the germinating power of poor seeds more than that of good ones. Conspicuous improvement under stratification was observed for several species which were making unusually low records from that particular harvest after dry storage; *e.g. Andropogon scoparius* in the first year, *Panicum virgatum* in the second, *Bulbilis dactyloides* during both seasons, *Achillea occidentalis* and *Silphium integrifolium* during the first test. *Helianthus rigidus* from the harvest of 1931 produced consistently the lowest germinations obtained from the species (from one to zero per cent). Stratified, however, the seeds of this crop gave the maximum for the species, 19.5 per cent. The harvest of 1933, on the other hand, produced a distinctly higher germination of seeds frozen in dry storage than the crop of any other season studied. This percentage (17) was not altered under stratification. *Physalis lanceolata*, ordinarily very prolific of seedlings, gave from the dry stored seeds of 1931 an extremely low record (20 per cent, contrasted with 48, 67, and 83 of the 3 previous years). The stratified seeds, however, from this apparently poor crop germinated 73 per cent. The supplementary test with seeds of 1933 showed improvement of similar magnitude over low germination of dry frozen seeds (from 12 to 69 per cent). The stimulus of cold for a period of 3 or 4 months on the well moistened seeds more than compensated, apparently, for the condition of development which they had attained at the time of harvest. Temperatures slightly above freezing (not actually freezing) are regarded as essential for the chemical and physical changes which characterize stratification (Davis and Rose, 1912; Eckerson, 1913). In most instances freezing does no harm.

Stratification produced more striking improvement in the seeds of forbs,

on the whole, than in those of grasses. The largest increases were in species which usually yielded very low percentages. The germinations from stratified seeds appeared in April and to a less extent in May, months which sometimes displayed improvements in the records of species producing few seedlings when planted in winter. The increase, however, was very small compared to that which followed stratification. Germination of *Verbena stricta* of 1931, which was 0.5 in both winter and spring tests and below 7 per cent for maximum germination during the 4 years, increased to 53 per cent after stratification. The repetition test of this species with the harvest of 1933 was less conspicuous. Dry freezing was followed by a germination of 7 per cent, stratification by one of 19.5 per cent.

One grass only underwent improvement comparable to that shown by several forbs. *Bulbilis dactyloides* produced out of the harvest of 1931 a germination of 6 per cent from seeds frozen dry and 35 per cent from stratification; out of the harvest of 1933 a germination of 3 per cent from dry freezing and 42 per cent from stratifying. The highest germination obtained during the entire study for this species and for *Sorghastrum nutans* and *Spartina michauxiana* came from stratified seeds. The remarkably large figures for Spartina from the 1933 crop (20 per cent without stratification, 46 per cent following it) were due to the selection of seeds for the presence of embryos by the aid of electric light, as described for the final germination test of *Agropyron smithii.*

Koeleria cristata after stratification of the seeds of 1931 gave very nearly the highest germination obtained for the species, 58 per cent. It greatly exceeded the yields from the test made in late winter. A test of the same harvest, however, made in early January, produced a germination of 63 per cent, almost entirely between 10 and 14 days after the sowing.

Stratified seeds of *Stipa spartea* of 1931 produced a germination of 9 per cent, surpassing that of seeds planted in the greenhouse 7 or 8 months after being harvested. The actual maximum, however, was from unfrozen seeds of the same crop. This largest yield was from a planting of 100 seeds in the garden June 20, shortly after they had been collected. After lying in the ground 7 to 8 weeks they began to germinate. In the following 6 weeks they produced a stand of 19 seedlings. In the season under consideration this would have been impossible except for artificial watering.

CONCLUSIONS

Stratification of seeds of grasses through the winter months resulted generally in higher germination than was otherwise obtained from a given crop. As the harvests of only one or two seasons were tested for the effect of stratification, the results were not entirely conclusive. The maximum germination of 3 to 5 harvests was obtained, for most species, from seeds frozen in dry storage.

Stratification of seeds of forbs resulted, for the majority of species tested, in maximum germination greatly in excess of the percentages from all harvests stored dry, either unfrozen or frozen.

Stratification makes germination more independent of the season of sowing and less sensitive to variations in external conditions (Joseph, 1929). Many fluctuations in environment, adequate to arrest development of seeds from dry storage, are no deterrents immediately after stratification. Sometimes seedling production after thorough ripening in dry storage was found to be as high as that which followed stratification. More often, however, the output of dry-stored seeds was reduced by one or several of the conditions operating at the time of the test.

Seeds kept permanently dry, at temperatures well above freezing, experience the same chemical and physical changes, before they cease to be viable, which constitute the advantages of stratification (Flemion, 1933). Seeds tested, in the course of this study, at intervals during six years of dry storage yielded, in several instances, higher germination than the same species of the current year, or the same species under stratification. The difficulty in planting a crop of dry seeds capable of high germination lay in determining the time when their maximum capacity could be realized.

As several recent investigators have pointed out, the advantages of stratification are often lost when the seeds are transferred to dry storage at relatively high temperatures. If the chemico-physical changes which must precede germination are reversible in nature, altered external conditions may readily cause the reestablishment of dormancy. The structures, single or combined, which are instrumental in preventing germination, differ with the kind of seed. Most often the obstruction has been found in some part of the seed coat. Davis (1930), working on the seeds of *Ambrosia trifida*, demonstrated that a cold, moist medium, surrounding the seed, increased the permeability of the nucellar membrane to oxygen. Subsequent exposure to dry heat reduced the permeability and resulted in secondary dormancy.

Seeds planted in moist soil and left out-of-doors during winter complete the changes which must precede germination more quickly than in dry storage. As they are in the soil in early spring, they are able to develop into seedlings before the external medium becomes so hot or so dry as to induce chemical activity of an opposing nature. Air-ripened seeds, after passing through the same changes, are less likely to be held in the stage which is essential for germination until external conditions become favorable for development.

There is not much danger that native seeds will germinate prematurely in the ground during unseasonable warm periods in winter. September or October plantings might germinate to some extent in the fall. To obtain early and abundant germination in a given area out-of-doors in the spring,

very late fall sowing would appear to be essential for most forbs and advantageous for most grasses.

Optimum Water Content of Soil for Germination

Tests were made to determine the degree of soil moisture most favorable for germination. Such a determination is practically useful in germinating seeds in the greenhouse and is suggestive in considering the factors influencing seedling production under natural condition.

METHODS

Selection was made of several grasses and forbs which grow in locations characterized by considerable differences in soil moisture. Seeds were planted only in soil of such water content as they would be likely to encounter in nature.

The soil was a silt loam with a hygroscopic coefficient of 7.6, a water-holding capacity of 55 per cent and a pH of 7.7. The degrees of constant water content used were saturation, and percentages which produced approximately 2/3, 1/2, and 1/3 saturation. In addition three species were tested in soil of somewhat less than 1/5 saturation. As a control, all species were planted in soil which was alternately well watered and allowed to become quite dry.

The tests were made in deep, open pans, covered with glass plates and sealed with vaseline. Two kilograms of dry soil were placed in each pan. The required amount of water was thoroughly mixed with the soil for low percentages of saturation. For higher percentages, part of the water was mixed with the soil. The remainder was added very gradually and the soil was allowed to stand 2 or 3 days before planting, in order that equilibrium might be attained. Otherwise the soil structure was destroyed and the air more or less completely displaced.

For each species, equal, measured amounts of seeds were planted in the soil of various degrees of water content. Somewhat over 100 seeds of large size and about 300 seeds of very small size constituted a planting. Germination was expressed by numbers of individual seedlings which appeared. The plantings were made early in May. The experiment was continued through a period of 6 weeks.

SPECIES TESTED

Eight species of grasses and 4 of forbs germinated in sufficient numbers to be considered. Two species of Andropogon, two of Bouteloua, *Elymus canadensis, Koeleria cristata, Panicum virgatum*, and *Spartina michauxiana* composed the list of grasses. The forbs were *Amorpha canescens, Petalostemon candidus, Salvia pitcheri*, and *Solidago glaberrima*. All were planted in soil of 1/3, 1/2, and 2/3 saturation and in soil which was alternately wet and dry. *Andropogon scoparius, Bouteloua curtipendula*, and *Amorpha canes-*

cens were planted also in soil of about 1/5 saturation. Four species of grasses and one forb were planted in saturated soil: *Andropogon furcatus*, *Elymus canadensis*, *Panicum virgatum*, *Spartina michauxiana*, and *Salvia pitcheri*.

RESULTS

One-third saturation proved to be distinctly the optimum for *Amorpha canescens*. *Petalostemon candidus* also germinated better in one-third saturation than in any other degree of constant water content. Its maximum germination, however, occurred in soil which was alternately wet and dry.

One-half saturation proved to be optimum for *Bouteloua curtipendula*, *Koeleria cristata*, and *Salvia pitcheri*. *Bouteloua curtipendula* produced considerable numbers of seedlings in all degrees of moisture, even one-fifth saturation. It was the only species which germinated in such dry soil. The seeds were from plants grown in a garden, as the harvest of this species secured on the prairie during the preceding summer yielded practically no germination. *Koeleria cristata* produced good germination in 1/3 saturation, while *Salvia pitcheri* gave none.

Two-thirds water content was optimum for 3 grasses: *Andropogon scoparius*, *Elymus canadensis*, and *Spartina michauxiana*. *Elymus canadensis* gave very poor germination for a species which usually grows so readily. Neither this species nor Spartina germinated under complete saturation.

Equally good germinations in both 1/2 and 2/3 saturation were recorded for *Andropogon furcatus*, *Panicum virgatum*, and *Solidago glaberrima*. *Andropogon furcatus* showed the most uniform germination of any species tested, judged by both numbers of seedlings, and latent periods. Even under saturation 2 germinations occurred at the end of 10 days. This was the only species which germinated in a saturated medium. *Panicum virgatum*, which usually produced a negligible percentage, gave a germination probably too small to be significant. The details of the numbers of seedlings and the latent period of greatest amount of germination in each group are given in Table 8.

DISCUSSION

The quality of the seeds must be taken into consideration in interpreting the results of such an experiment. The inferiority of seeds raised under adverse conditions may permit them to germinate only where water content is optimum. The superior osmotic and imbibitional power of well developed seeds may considerably extend their range of germination. The optimum for a species would probably be constant. The limits from which germination might be expected would vary with the harvest.

The maintenance of a proper balance between water supply and oxygen supply determines the water content of soil which is optimum for germination. Alternate wetting and drying improve aeration over that of soil of constant moisture. The danger lies in the probability that, after a seed has

TABLE 8. Relation of germination to soil moisture

Species	Germination	1/3 sat.	1/2 sat.	2/3 sat.	Alternately wet and dry
Andropogon furcatus	No. of plants	6	9	10	8
	Time (days)	7	7	7	10
Andropogon scoparius	Plants	15	16	18	11
	Time	13	11	11	20
Bouteloua curtipendula	Plants	65	81	37	49
	Time	14	5	5	9
Bouteloua gracilis	Plants	4	4	3	5
	Time	8	4	4	7
Elymus canadensis	Plants	1	7	16	9
	Time	24	27	10	24
Koeleria cristata	Plants	161	261	59	25
	Time	8	8	4	14
Panicum virgatum	Plants	0	1	1	1
	Time	..	7	7	7
Spartina michauxiana	Plants	0	0	3	1
	Time	..	..	37	31
Amorpha canescens	Plants	92	36	17	22
	Time	6	6	6	6
Petalostemon candidus	Plants	31	27	6	44
	Time	10	4	4	7
Salvia pitcheri	Plants	0	21	14	17
	Time	..	4	4	10
Solidago glaberrima	Plants	0	5	5	0
	Time	..	4	4	..

initiated germination and its protoplasm has been converted into the unstable, sensitive state, subsequent drying will injure it. It may be killed, or it may merely be reduced again to a dormant condition. In either event no seedling will be produced. When a seed germinates quickly enough to avoid drying, it takes the first step in increasing the establishment of its species. Whether or not young plants will survive long enough out-of-doors to be seen, even if they have succeeded in germinating, will depend on the rapidity with which their seminal roots penetrate the generally moister layers below the level of the seed.

CONCLUSIONS

Two-thirds saturation was the optimum constant water content for germination for species characteristic of the more moist parts of the prairie. One-third to one-half saturation was the optimum of constant water content for most species of dry areas. For species which germinated very quickly, the optimum was sometimes furnished by alternate wetting and drying.

GERMINATION IN PRAIRIE SOD

Germination in prairie sod kept under favorable conditions of temperature and moisture in the greenhouse was studied during 4 years, from 1928 to 1931. Half of the lot of sod was frozen out-of-doors before germination was permitted to occur.

METHODS

Sod was removed from the prairie about November 1, after most of the seeds had fallen but before the ground became frozen. It was cut to a depth of 4 inches and fitted into flats carried into the field. The surface area was about 20 square feet, except that the amount collected the first year was considerably smaller. Two flats were filled at each location selected, so that duplicate sets were obtained. One of these was placed in the greenhouse immediately and kept under conditions favorable to germination. The other was well watered, covered with cheesecloth to prevent the entrance of seeds, and left out-of-doors until the middle of February. It was then brought into the greenhouse to induce germination. Inspection was made at intervals of 2 to 4 weeks for the appearance of seedlings.

All collections were from the interior of a square mile of unbroken prairie 9 miles northwest of Lincoln, except a part of the samples taken in 1928. These were from the less isolated Belmont prairie, directly north of Lincoln, and afforded the large number of weedy grasses which appeared during the first year.

RESULTS

Germination varied widely from year to year both in species and in numbers of individuals. Only 4 grasses and 4 forbs were represented during each of the 4 years. These were *Koeleria cristata*, *Panicum scribnerianum* Nash, *Poa pratensis*, *Erigeron ramosus*, *Linum sulcatum*, *Oxalis stricta*, and the ruderals, *Panicum capillare* and Lepidium spp. Five other grasses and 3 forbs appeared during each of 3 years. These included *Andropogon furcatus*, *Elymus canadensis*, *Festuca octoflora* Walt., *Senecio plattensis*, and the weedy plants, *Bromus tectorum* L., *Syntherisma sanguinalis* (L.) Dulac, Euphorbia spp., and *Silene antirrhina* L. The number of individuals was never large except for the 4 grasses, *Bromus tectorum*, *Festuca octoflora*, *Koeleria cristata*, and *Poa pratensis*, and the one forb *Erigeron ramosus*.

Seedlings of 22 species of grasses appeared during the 4 years. They included *Andropogon furcatus*, 10 plants; *A. scoparius*, 1; *Aristida oligantha*, 2; species of Bouteloua, 6; *Elymus canadensis*, 13; *Festuca octoflora*, 344; *Koeleria cristata*, 613; Muhlenbergia sp., 7; *Panicum scribnerianum*, 67; *Poa pratensis*, 4,437; *Sorghastrum nutans*, 1; and *Stipa spartea*, 1. A considerable number of weedy grasses germinated. Of these only *Bromus tectorum* produced a large number of seedlings (259). Twelve seedlings of sedges and over 20 of rushes were found.

Fifty-one species of forbs were represented. The largest number, 29 species, germinated in sod obtained in the fall of 1930. Annuals included *Androsace occidentalis* Pursh, 5 specimens; *Hedeoma hispida* Pursh, 6; *Linum sulcatum* Riddell, 13; *Polygala verticillata* L., 7; *Silene antirrhina* L., 45; and *Specularia perfoliata* (L.) A. DC., 10. Typical perennials in-

cluded *Anemone cylindrica*, Nutt., seedling; *Astragalus crassicarpus* Nutt. (*Geoprumnon crassicarpum* (Nutt.) Rydb.), 3; *Comandra umbellata* (L.) Nutt., 3; *Erigeron ramosus*, 352; *Fragaria virginiana* Duchesne, 1; *Galium aparine* L., 2; *Helianthus rigidus*, 3; *Kuhnia glutinosa*, 2; species of Lithospermum, 3; Meibomia sp., 1; *Oenothera biennis*, 1; *Oxalis stricta* L., 110; species of Petalostemon, 10; Physalis sp., 1; species of Psoralea, 24; species of Solidago, 5; *Steironema ciliatum* (L.) Raf., 3; *Verbena stricta*, 2; *Vernonia baldwini*, 8; and *Viola pedatifida* G. Don, 9. From 10 to 15 species belonged to the group of ruderals; *e.g.*, *Ambrosia artemisiaefolia* L., 1 seedling; species of Brassica, 2; *Bursa bursa-pastoris* Brit., 3; *Chenopodium album* L., 3; species of Lactuca, 5; species of Lepidium, 48; *Leptilon canadensis* (L.) Brit., 12; *Rumex altissimus* Wood, 2; species of Sisymbrium, 6; and *Taraxacum taraxacum* Karst., 3.

DISCUSSION

In contrasting the germinations in frozen and unfrozen sod, it was impossible to determine whether the failure of a species to appear in one of the duplicates was due to exposure or protection in the matter of freezing or to the absence of seeds. Few species were represented by sufficient numbers to be dependable indicators of the effect of freezing.

A few examples of apparent benefit from freezing may be cited. The total number of Festuca seedlings was 245 from the frozen sod and 99 from that which had not been frozen. *Panicum scribnerianum* and *Oxalis stricta* consistently gave more germination in frozen than in unfrozen sod. For the former the respective yields were 52 and 15; for the latter, 89 and 21. *Andropogon furcatus* produced 10 seedlings during the 4 years. Nine were from sod which had been frozen. This was in agreement with the higher percentage of germination of seeds which had been frozen in dry storage, compared to the unfrozen seeds of the species, in the regular winter tests.

The reverse effect is suggested by the record of Koeleria. Each year the unfrozen sod had more seedlings than the frozen duplicate, with totals of 453 and 160 respectively. Germination tests, on a percentage basis, indicated slightly better germination from frozen seeds. *Poa pratensis* showed the same tendency. With the exception of one year, the seedlings were more abundant in unfrozen sod (3,614) than in frozen sod (823). Over 3,000 of the unfrozen seeds germinated in the sod of 1930. The largest number of seedlings of both these prolific species appeared in November. Tests of frozen and unfrozen seeds did not support this result. Probably it was due to the presence of large numbers of seeds of poor quality. Such seeds were able to germinate for a few weeks. They lost their vitality before the frozen sod was exposed to conditions conducive to development. *Erigeron ramosus*, with the exception of one year, produced somewhat more seedlings in unfrozen than in frozen sod, 188 as against 164. The observation of many

seedlings of Erigeron was made in the prairie each fall before frost. It was not certain whether they came from the unfrozen seeds which had fallen during the summer, or were developed from seeds which had lain in the ground through the previous winter. Of 5 specimens of Androsace found in two years, all except one were from unfrozen sod.

Seedlings of typical prairie species germinate in greater numbers under greenhouse care than under natural conditions. Large areas of prairie must be searched to yield the variety and number of individuals found in the surface area employed in the experiment. Absence of favorable combinations of heat and moisture, or sudden drying of a seed when germination has progressed to a critical stage are the chief causes of death of viable seeds before they can produce seedlings. Destruction by animals also is greater out-of-doors.

CONCLUSION

Relatively few of the viable seeds which fall on the prairie germinate under natural conditions.

GERMINATION IN THE PRAIRIE

Search was made for seedlings in the spring of the 4 years of 1929 to 1932. Similar observations were made in the fall in 1929 and 1931. The results of this study are qualitative rather than quantitative, as relatively large areas had to be inspected in order to obtain a variety of species or an appreciable number of those which were more abundant.

METHODS

Quadrats 35 centimeters square were located at random over considerable areas of prairie. Any seedlings present were listed. Unless they were to be studied later, young plants without cotyledons were excavated to make certain that the shoot had not arisen from an old plant.

DISCUSSION

Immediately after warm rains, as during the charting of some quadrats in July, 1929, the numbers of seedlings found were much larger than usual. Subsequent investigation showed that such germination amounted to very little, if any, permanent establishment. Only by germination in spring or fall do seedlings ordinarily have a chance to escape destruction by summer drought. After the first summer's observation, search for seedlings was not made after the middle of June or before the middle of October.

Most of the species which occurred in significant numbers were found in both spring and fall. *Meibomia illinoensis*, however, represented by a total of 17 seedlings during 4 years, was seen only in the spring and was found during each spring of the study. *Kuhnia glutinosa*, *Liatris punctata*, and Helianthus sp. were found exclusively in the spring, and occurred in very

small numbers during only two years of the four. Accordingly, the results from the observations of the 2 seasons have not been considered separately, but have been grouped by years.

RESULTS

Relatively few seedlings were found, although during the 4 years 42 species of forbs and 6 of grasses were observed. For any single season the number was small. Six species of forbs were seen each year: *Erigeron ramosus*, *Lactuca ludoviciana* (Nutt.) DC., *Meibomia illinoensis*, *Oxalis stricta*, *Senecio plattensis* Nutt., and at least one species of Solidago. Four other species seen during 3 years were Lepidium sp., *Linum sulcatum*, *Melilotus alba* Desv., and Petalostemon sp. The list of forbs recorded during 2 years included *Achillea occidentalis*, *Amorpha canescens*, *Anemone cylindrica*, *Aster multiflorus*, Carduus sp., Helianthus sp., *Kuhnia glutinosa*, *Liatris punctata*, *Lithospermum arvense*, Physalis sp., *Psoralea floribunda*, *Specularia perfoliata*, and species of Viola. Seedlings of two shrubs, *Rhus toxicodendron* L. (*Toxicodenedron rydbergii* (Small) Greene), and *Rosa arkansana* were also seen during 2 years.

Very few kinds of grass seedlings were observed. Of a total of 6 species, only *Panicum scribnerianum* was found each year. *Festuca octoflora*, *Koeleria cristata*, *Panicum capillare*, and *Poa pratensis* were all present during two years. A species of Andropogon was observed once.

During 1929 a total of 583 seedling forbs was found, comprising 22 species. Four of them were ruderals. Of typical prairie plants, *Erigeron ramosus* furnished 391 seedlings; *Achillea occidentalis*, 38; *Senecio plattensis*, 25; *Oxalis stricta*, 22; *Aster multiflorus*, 17; *Meibomia illinoensis*, 8; *Anemone cylindrica*, 6; *Antennaria campestris*, *Artemisia gnaphalodes*, and *Echinacea pallida*, 5 each; Solidago sp., 4; and other species even smaller numbers. Among the grasses *Koeleria cristata* was represented by 133 seedlings; *Festuca octoflora* by nearly a hundred; *Panicum scribnerianum* by 7; and *P. capillare* by 3.

In 1930 only 8 species of forbs were recorded. *Erigeron ramosus* again furnished the largest proportion, 8 plants of the total list of 17. Solidago sp. and *Specularia perfoliata* were represented by 2 seedlings, other species by only one. Eighty seedlings of *Koeleria cristata* and 34 of *Panicum scribnerianum* composed the grasses.

In 1931 thirty species and 249 individuals comprised the forbs. *Erigeron ramosus* again headed the list with 51 plants. If the ruderals are disregarded, the more abundant representatives are *Oxalis stricta*, with 45 seedlings; *Psoralea floribunda*, 23; *Salvia pitcheri*, 18; *Lithospermum arvense*, 16; *Linum sulcatum*, 13; *Oenothera biennis*, 6; Physalis sp., 8; *Senecio plattensis*, 6; 5 each of *Baptisia bracteata* A. Gray and *Meibomia illinoensis;* 4 each of *Hedeoma hispida* and Viola sp.; and 3 each of *Amorpha canescens*,

Helianthus sp. and also of the shrub *Rhus toxicodendron*. The list of grasses included 5 species and 46 seedlings. These were *Poa pratensis*, 20 plants; *Panicum scribnerianum*, 10; *P. capillare*, 8; *Festuca octoflora*, 7; and one of a species of Andropogon.

In 1932 twenty-two species of forbs and 127 seedlings were found. They included *Erigeron ramosus*, 32 plants; *Specularia perfoliata*, 30; *Anemone cylindrica*, 7; *Psoralea floribunda*, 6; *Vernonia baldwini*, 5; 3 each of *Lithospermum arvense* and *Meibomia illinoensis;* and 2 each of *Amorpha canescens*, *Liatris punctata*, *Oxalis stricta*, Petalostemon sp., *Scutellaria parvula* Michx., *Senecio plattensis*, and Solidago sp. Four seedlings of *Panicum scribnerianum* and 3 of *Poa pratensis* composed the record of grasses.

Where subsequent investigation of seedlings was possible, establishment was much less common than destruction.

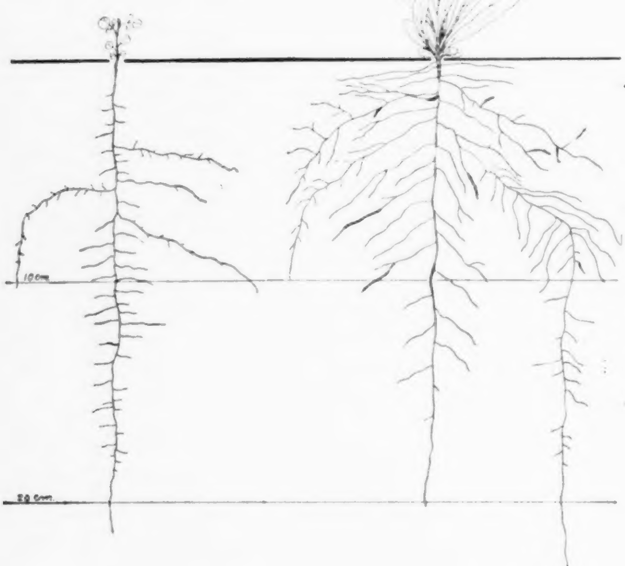
CONCLUSION

Except for the relatively few annual and short-lived plants, germination resulted in small numbers of widely scattered individuals, which frequently failed to survive.

EARLY LIFE HISTORY OF PRAIRIE PLANTS

After germination the seedling is under the immediate necessity of developing a root system and a foliage surface which will make it self-supporting when the supplies stored in the seed or cotyledons are consumed. The root grows rapidly and penetrates the deeper soil which remains permanently moist (Fig. 2). Unless it is able to accomplish this, the development of the entire plant will be slow. Low water content necessitates a considerable area of absorbing surface to furnish water at the rate demanded by the growing top. If drought overtakes the young plant, the shoot is unable to elongate and the leaves are small and lack turgidity. Photosynthesis, in the deepening shade of the established vegetation, becomes impossible. The result is death by starvation. Contact with the supplies of water in the deeper soils is effected promptly in the normal development of seedlings. This enables the seedling which germinated in the spring to live through the heat and occasional drought of summer. It assists the seedling which germinated in the fall to accumulate in its relatively limited tissues the reserve materials which maintain its diminished metabolism throughout the winter.

The rate of development has been studied in both controlled and natural environments. Two aspects of growth under optimum conditions have been considered. The development of roots in relation to tops was investigated in the greenhouse. The development of shoots of seedlings in a garden was followed throughout the growing season. To determine the early history in nature, seeds were planted in the prairie in the spring and the fate of the seedlings was observed throughout the summer. Seedlings which had germi-



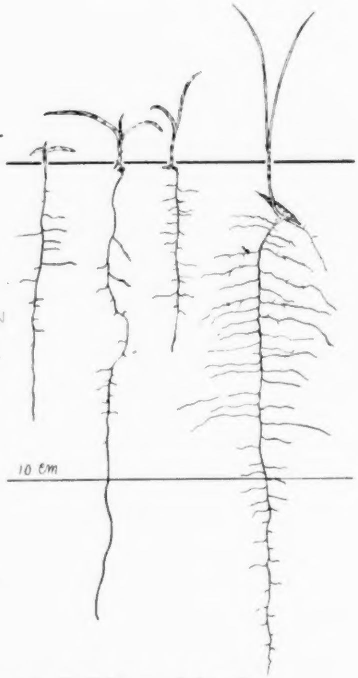
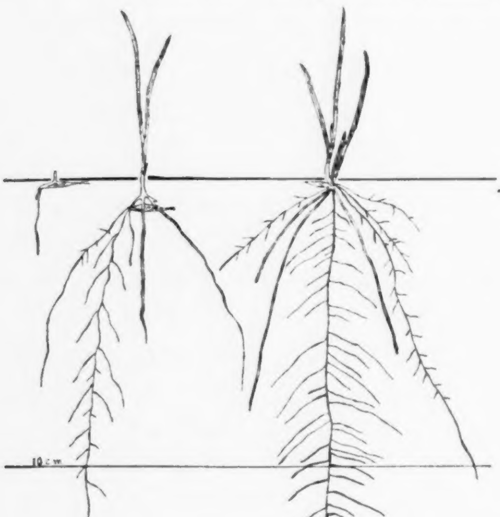


Fig. 3. Seedlings of *Amorpha canescens* (left) and *Solidago glaberrima*, about 6 weeks old.
Fig. 4. Early development of grass seedlings: two stages of *Panicum virgatum* (left); *Bouteloua curtipendula* (center); and *Stipa spartea* (right).



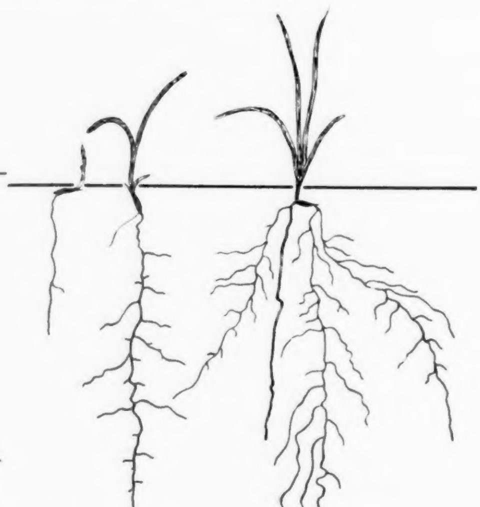


Fig. 5. Three stages in the development of *Elymus canadensis:* seedlings one, thirteen, and seventeen days old.
Fig. 6. Three stages in the development of *Andropogon furcatus:* seedlings three, eleven, and twenty-two days old.

nated naturally in the fall were located in late autumn and inspected again in early May.

DEVELOPMENT OF ROOTS IN RELATION TO TOPS

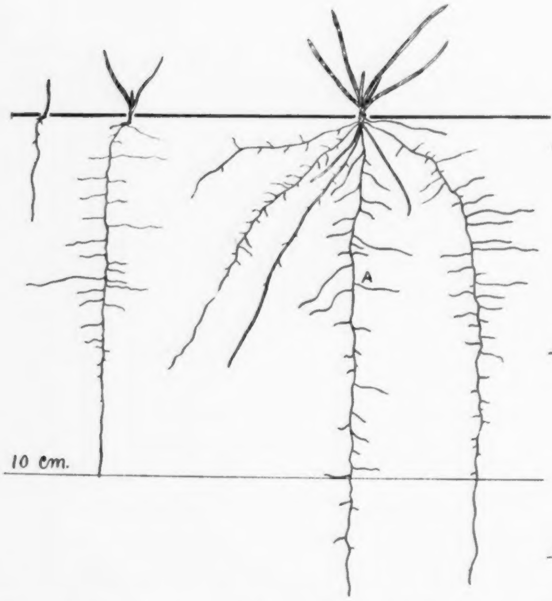
In soil uniformly supplied with water, oxygen, and nutrients, species show characteristic habits of growth. *Solidago glaberrima*, for example, possesses a diffuse root system with a wide lateral spread immediately below the soil surface but *Amorpha canescens* produces a strong taproot with short laterals growing out at right angles (Fig. 3). Only after study of control plants is it possible to evaluate the influence of environment. The root systems of young seedlings have been investigated for a considerable number of grasses and forbs.

METHODS

The seedlings were grown in a specially constructed series of wooden boxes. The depth of each box was 20 cm. and the surface area of the interior was 20 by 20 cm. One side was attached by means of brass screws, so that it could be removed. The boxes were filled with soil of good tilth, firmed but not packed. This permitted growth to take place equally well in any direction through the soil. Watering was done sparingly.

RESULTS

Rapid increase in absorbing surface was illustrated by all of the grasses (Fig. 4). The root makes considerable elongation before the shoot appears above ground, sometimes even before it escapes from the seed coat. In *Elymus canadensis* the laterals of the seminal root form early and branch widely (Fig. 5). *Andropogon furcatus* is a much more mesic species, characterized at maturity by great depth of root penetration (Weaver, 1919). This feature is foreshadowed in the early elongation of its seminal roots and the unusually prompt development of a conspicuous secondary root which grows directly downward (Fig. 6). *Koeleria cristata*, a species of much more shallow root habit, shows more extensive branching close to the surface than either of the bluestems (Fig. 7). The secondary root system of many of the grasses, such as *Koeleria cristata*, *Bouteloua gracilis*, and *Stipa spartea*, begins to appear at the time of tillering or shortly before (Fig. 8). Subsequent tillers are accompanied or slightly preceded by other adventitious roots. This is illustrated by the densely branched system of *Sorghastrum nutans* and the deeply but more sparsely rooted *Panicum virgatum* (Fig. 9). Weaver, Kramer, and Reed (1924) reported similar findings in the case of winter wheat. Under favorable conditions, organs of vegetative propagation form very abundantly during the first year. A spring seedling of *Spartina michauxiana*, by late October, had developed 27 large rhizomes from 2 to 10 cm. in length (Fig. 10). Corresponding development in forbs was seen in a specimen of *Achillea occidentalis* of the same age.



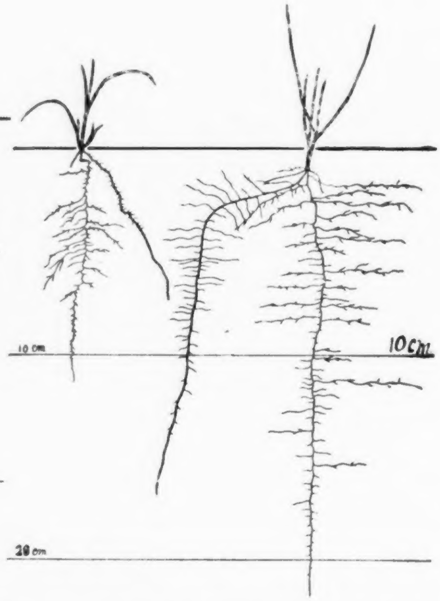
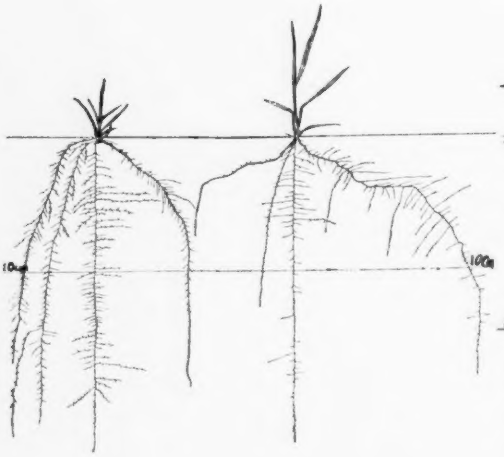


FIG. 7. *Koeleria cristata*, 4, 20, and 31 days after germination. The absorbing area afforded by the seminal root (A) has been greatly augmented by the development of several adventitious roots.

FIG. 8. Development of tillers and adventitious roots in *Bouteloua gracilis* (left); and *Stipa spartea*, between 4 and 5 weeks old.



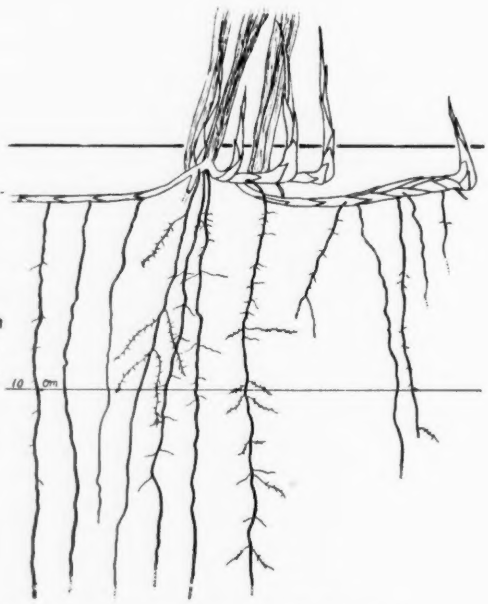


FIG. 9. Adventitious roots and tillers of *Sorghastrum nutans* (left) and *Panicum virgatum*, about 6 weeks old.

FIG. 10. Portion of *Spartina michauxiana* at the end of the first summer, showing abundant rhizomes and coarse roots.

Native dicotyledonous plants show the same rapid development of roots compared with tops, as is illustrated by *Oenothera biennis* (Fig. 11). An unbranched taproot is commonly extended to a depth of several centimeters while the cotyledons are unfolding. Such composites as *Liatris punctata* and *Kuhnia glutinosa* also demonstrate this phenomenon (Fig. 12). In Liatris, growth below ground is much more significant than expansion of the top during the entire first year. While a single narrow leaf represents the shoot for a long time, thickening of the taproot in the region immediately below the ground level becomes apparent very early. Even under partial shade and adequate water supply in a garden, development of the shoot was confined to a basal rosette but storage below the soil was pronounced in June. It resulted by late October in an enlargement nearly a centimeter in diameter. *Kuhnia glutinosa,* a species which is capable of fruiting during its first season, developed more leaf surface in comparison with the young root system. *Antennaria campestris,* a species of wide distribution in the prairie, is found most abundantly in areas of thin or sandy soil. As it is prevernal it is exposed for a long time to full sunlight. Like *Koeleria cristata* among the grasses, it branches widely in the upper soil (Fig. 13). The leaves remain small and few while the absorbing organs are prolonged as a taproot with extensive laterals.

CONCLUSIONS

Upon germination plants of the prairie rapidly developed a deeply penetrating root which often has lateral branches before much of the shoot is exposed to water loss. In the grasses tillering begins at the same time as the development of the secondary root system. Both grasses and forbs frequently develop rhizomes or other off-shoots after only a few weeks of growth, while continuing their root formation with equal rapidity. During all stages of development, growth of foliage is relatively slow and small compared to that of underground parts.

SHOOT DEVELOPMENT OF SEEDLINGS IN A GARDEN

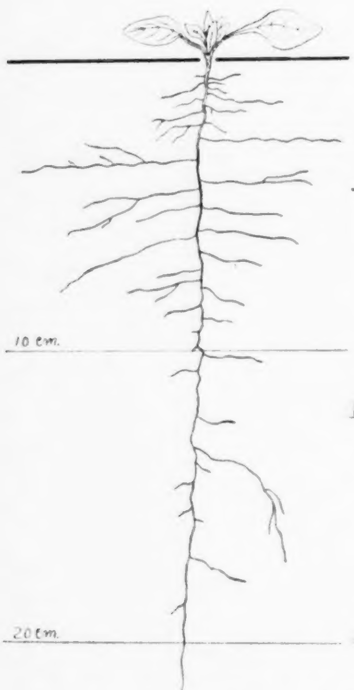
Several species of perennial grasses and forbs were grown in a garden to determine their development under favorable conditions during a single summer.

METHODS

The seeds were planted in pots in the greenhouse about the middle of April. The seedlings were transplanted to the garden on May 15. The soil was well watered and kept free from weeds until the middle of August. For a time the seedlings were protected from direct sunlight for several hours each day. The crop of seeds obtained was tested for germination.

RESULTS

Of 14 species of grasses, 9 produced viable seed the first year. They were *Andropogon furcatus, Aristida oligantha, Bouteloua curtipendula, B.*



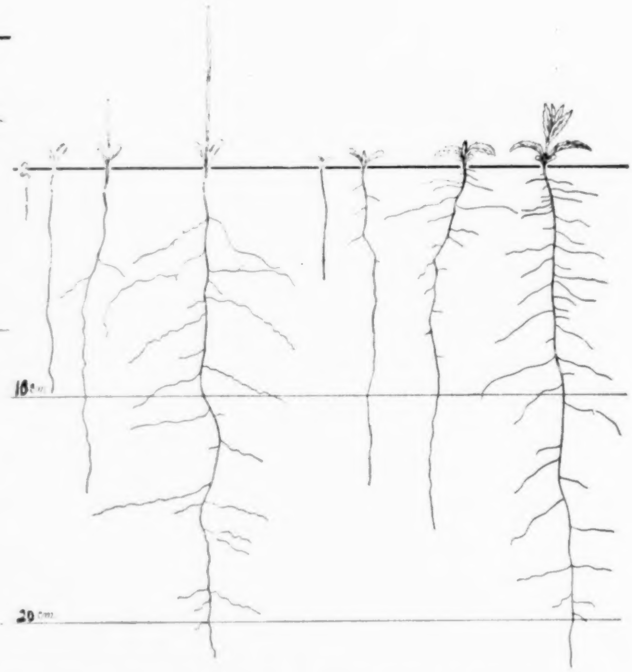
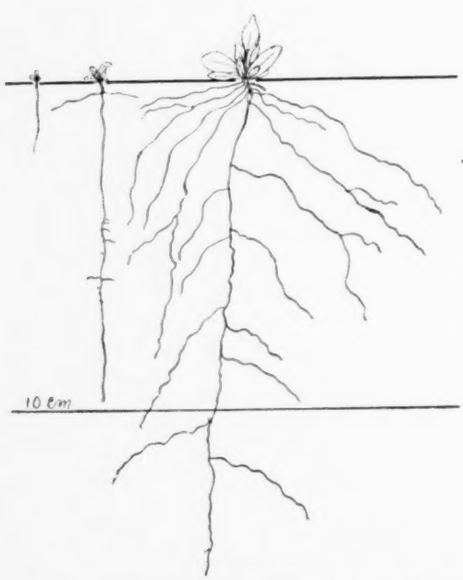


FIG. 11. Seedlings of *Oenothera biennis* showing single pair of leaves unfolded and taproot.
FIG. 12. Seedlings of *Liatris punctata* (left) 1, 4, 8, and 16 days after their appearance above ground, and seedlings of *Kuhnia glutinosa* of similar ages.



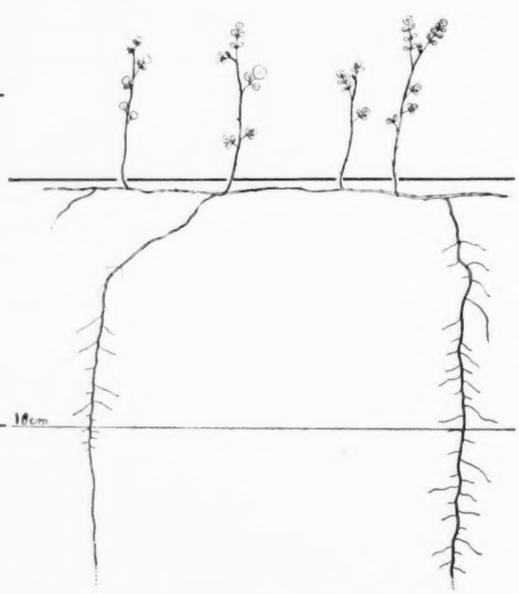


FIG. 13. Stages in the growth of *Antennaria campestris* showing the early development of an extensive surface absorbing system. The plants are 12, 24, and 56 days old, respectively.
FIG. 14. *Amorpha canescens* in early June, showing vegetative propagation from rhizomes.

gracilis, *B. hirsuta*, *Panicum virgatum*, *Sorghastrum nutans*, *Spartina michauxiana*, and *Sporobolus asper*. The seeds of 3 species gave remarkably high germination: *Andropogon furcatus*, 23 per cent; *Aristida oligantha*, 75 per cent; and *Bouteloua curtipendula*, 30 per cent. The germination from *Bouteloua hirsuta* was less than one per cent. *Elymus canadensis* formed heads which appeared normal but no seeds were present. Good vegetative growth but no flower stalks were obtained from *Agropyron smithii* and *Stipa spartea*. *Andropogon scoparius* failed to become established after being transplanted.

Of 21 perennial forbs, only 6 were induced to bloom during the first year and viable seeds were produced by only 3, *viz.*, *Kuhnia glutinosa*, *Physalis lanceolata*, and *Vernonia baldwini*. *Oenothera biennis* fruited freely but no germination was obtained from its seeds. *Achillea occidentalis* flowered in October, too late to form seed. *Salvia pitcheri* blossomed abundantly but the branches were accidentally broken before the seeds ripened. In all of these species the stature of normal, mature plants was attained.

Antennaria campestris and *Astragalus canadensis* died early in the summer from lack of sufficient protection from the sun. *Aster multiflorus* formed only a basal rosette. *Amorpha canescens* and Petalostemon sp. were continually injured (possibly by rabbits) and did not reach 10 cm. in height. *Lespedeza capitata* grew to 15 cm. and was decapitated in August. *Liatris punctata* and *L. scariosa* formed only basal rosettes, but excellent storage organs. Clements and Weaver (1924) have brought these species to fruit in one season. *Solidago rigida*, *Silphium integrifolium*, and *S. perfoliatum* L. produced large basal rosettes. In the second spring the two rosin-weeds resumed growth early and suffered little harm from subsequent freezing weather. They fruited heavily in the summer. The ruderal *Tragopogon pratensis* made only foliage growth.

CONCLUSIONS

Many of the long-lived prairie species, which do not bloom ordinarily until their second or third year are capable, under favorable conditions, of forming normal amounts of foliage and viable seed during the first year.

SEEDLINGS IN THE PRAIRIE

In tracing the history of seedlings of perennials, the first difficulty is to make certain that the young shoots arise from seeds rather than from underground parts of established plants. Unless cotyledons or seed coats are found, the proof, in most cases, depends upon following the root system until a clear demonstration is afforded that the plant originated from a seed of the current season. *Helianthus rigidus*, for example, initiates growth each spring with leaves which can scarcely be distinguished from those of seedlings. The majority of apparent seedlings arise by vegetative propagation

from older plants (Fig. 14). Further, the seedlings found growing in the prairie are nearly always so few and so widely scattered that they do not form an adequate basis for recording the development of most species (Steiger, 1930). In order to obviate these difficulties in studying the establishment of native seedlings, seeds were planted in undisturbed prairie.

Growth begins to be generally apparent on the prairie early in April. It consists almost entirely of new shoots from established vegetation and normally seedlings do not appear in abundance until 3 to 4 weeks later.

The seeding was done at Belmont prairie, directly north of Lincoln, early in April of two consecutive years, 1930 and 1931. Plantings were made in two types of prairie: upland, along the top of a small ridge; and lowland, the nearly level ground at the foot, at a distance of about 50 feet from the upland station. *Andropogon scoparius* dominated the upland but there was also a considerable mixture of *Koeleria cristata* and some *Andropogon furcatus*. The grass of the lowland was an almost pure stand of *Andropogon furcatus*.

METHODS

Two types of seeding were used, surface sowing and shallow planting. For surface sowing, the soil was loosened with the blade of a small knife. The seeds were sprinkled into the debris and slightly mixed with it, to prevent blowing and to simulate the effect of planting by nature in autumn. For shallow planting, the ground was broken and the seeds were placed in it and covered to a depth of one-fourth to one-half inch, depending upon their size.

On both upland and lowland two lines of planting were made, one of seeds sowed on the surface and one of shallowly planted seeds. A small stake at the place of planting of each species made it possible to relocate the seedlings.

The seeds were from the harvest of the preceding fall. They had been stored dry and frozen out-of-doors during the winter. Approximately equal, measured quantities of seeds of each species were used for each planting at both stations. The unit was somewhat over 100 seeds except when high germination could be expected; then the number was reduced to about 50.

The seeds were planted early in April. Examination for germination and development was made at intervals of about one week until the middle of May, and on alternate weeks after that time. Inspection was discontinued only after the advent of killing frost. Search for winter survival was conducted during the following spring.

Soil moisture was determined at depths of 0 to 6 and 6 to 12 inches during very dry periods in July, when conspicuous numbers of seedlings were dying. Light intensity among the seedlings was determined at inter-

vals. Access was also had to hygrothermograph, anemometer, and atmometer records at a station of a co-worker only a few rods distant.

SPECIES TESTED

Seventeen species of grasses were tested during the two years. Fourteen of these were planted both years. They included the prairie dominants, *Andropogon furcatus*, *A. scoparius*, *Bouteloua curtipendula*, *Elymus canadensis*, *Koeleria cristata*, *Panicum virgatum*, *Sorghastrum nutans*, *Sporobolus heterolepis* A. Gray, *Stipa spartea*, and *Sporobolus asper*. Two typical, low-growing grama grasses, *Bouteloua gracilis* and *B. hirsuta*, were planted both years, and the preclimax *Aristida oligantha*, which usually occurs along the edge of the prairie as a ruderal. *Bulbilis dactyloides* and the ruderal grasses, *Chaetochloa glauca* and *Panicum capillare*, were tested during one year only. *Carex festucacea* Schkuhr. was planted each year.

Thirty species of forbs were planted, 22 of them during both years. They included *Achillea occidentalis*, *Amorpha canescens*, *Antennaria campestris*, *Artemisia gnaphalodes*, *Aster multiflorus*, *A. salicifolius*, *Astragalus canadensis*, *A. crassicarpus*, *Echinacea pallida*, *Gaura parviflora* Dougl., *Glycyrrhiza lepidota*, *Helianthus rigidus*, *Kuhnia glutinosa*, *Lespedeza capitata*, *Liatris punctata*, *L. scariosa*, *Meibomia illinoensis*, *Oenothera biennis*, species of Petalostemon, *Physalis lanceolata*, *Salvia pitcheri*, *Silphium integrifolium*, *S. laciniatum* L., *Salidago canadensis* L., *S. rigida*, *Verbena stricta*, and *Vernonia baldwini*. *Grindelia squarrosa*, often seen in this region in overgrazed prairie, and the ruderals, *Helianthus annuus* and *Tragopogon pratensis*, were also planted.

CONDITIONS FOR GROWTH

Cool weather marked the beginning of the growing season of 1930. The soil was well supplied with water and the air was quite humid during the spring and early summer. From the middle of June until the second week of August, extreme heat and drought characterized the season. Considerable rain fell in the last half of August. September was warm and dry. In 1931 the spring was relatively dry.

The rain of both May and June fell in brief, heavy showers. June was very hot. The most sustained season of drought occurred during the first half of September.

The seeds planted in 1930 were from a harvest which yielded a much higher percentage of germination than the seeds planted in 1931. The latter were collected after the exceptionally hot, dry summer of 1930. The germination in the field in 1930 was somewhat higher than that of 1931 in respect to number of species, and considerably higher in number of individuals. In 1931 the lowland seedlings suffered a casualty which was not normal to the prairie. They were situated where, under unusually heavy precipitation,

they received the wash from a cultivated field. Torrential showers late in May resulted in the burial of the seedlings of more than 50 per cent of the species which had germinated.

RESULTS

Differences from the two types of seeding were less apparent in the greater soil moisture during the spring of 1930 than during the following year. More seedlings were produced at both upland and lowland stations from planted seeds than from surface sowing. The differences, however, were seldom large. Sometimes a species, at one station or the other, gave a higher germination from surface sowing. On the whole, however, pushing up through the soil was less of a handicap than contact with the drier air above ground. No appreciable difference in time of appearance was detected. Since inspection was made at intervals of one to two weeks, the actual dates of germination were somewhat earlier than the recorded dates and small differences in time of germination were not seen. After the seedlings appeared above ground, their growth was not affected by the type of planting. The plants obtained from both methods of sowing have, accordingly, been considered as forming one group for each species at both the upland and the lowland stations.

No permanent establishment was obtained during either year. A small number of species, represented by a few individuals, survived until late summer. Before the following spring, however, and sometimes before the frosts of autumn, these perished.

Some of the plantings, especially in the drier soil in the spring of 1931, produced so few seedlings that a study of the species had to be based on very small numbers of individuals. As isolated plants frequently attained better growth than seedlings closely grouped, the development of even one or two plants has been permitted to serve as a record for the species.

Eighty-eight per cent of the grasses planted in 1930 germinated; *i.e.*, 15 species of a total of 17. Fifty per cent of 16 species germinated in 1931. *Sorghastrum nutans* grew only at the upland station. *Bulbilis dactyloides* and *Sporobolus heterolepis*, each planted one year only, failed to give any germination. The other species were represented at both stations. There was always a possibility that a species germinated and perished before any record was obtained. As the study was made primarily to determine establishment, such an omission was not extremely important.

Three species were present on September 30 of the first year: *Elymus canadensis*, *Koeleria cristata*, and *Sporobolus asper* in the lowland and *Elymus canadensis* (germinated after the summer drought) in the upland. Each was represented by 3 or 4 plants, under 10 centimeters high, bearing less than 5 leaves. The following spring only Elymus in the lowland was present. The 3 little plants were buried later under debris. None of the seedlings

of 1931 survived through August. A sedge, *Carex festucacea*, failed to appear during either year.

Between 70 and 75 per cent of the 25 to 27 species of forbs germinated each year. The upland only produced seedlings of *Antennaria campestris*, *Aster salicifolius*, *Meibomia illinoensis*, *Oenothera biennis*, and *Verbena stricta*. *Astragalus crassicarpus* appeared only in the lowland. No germination was obtained from *Artemisia gnaphalodes*, *Aster multiflorus*, *Glycyrrhiza lepidota*, and the two species of Solidago. Germination was given by one Solidago a year after it had been sowed.

By September 30 of the first year 4 species were present: *Achillea occidentalis* in the lowland; *Amorpha canescens*, *Kuhnia glutinosa*, and *Lespedeza capitata* on the upland. The maximum height was 9 centimeters. None was found the following spring. The second year the last species to succumb were *Helianthus rigidus* on the upland and *Salvia pitcheri* at both stations. They attained a maximum height of 16 centimeters before they died in severe drought in September.

Seeds which germinated a year after being sowed included species of Amorpha, Astragalus, Echinacea, Lespedeza, Meibomia, Oenothera, Petalostemon, Salvia, Solidago, and Vernonia. Only Lespedeza survived until August. It was dead by November.

CAUSES OF DEATH

Several factors combined to produce this wholesale mortality of seedlings. Relatively long periods of drought are normal to the climate. They constituted the most conspicuous cause of death. While July was exceptionally hot in 1930 and June was unusually hot in 1931, the two summers were in general more typical of the growing season in Nebraska than years characterized by high rainfall. Destruction by insects and allied animals is a contributing cause to the disappearance of seedlings. Its toll of young plants is mainly exacted so soon after germination that many of them completely escape the notice of the observer. A fundamental factor in the failure of perennial seedlings to establish themselves in their native prairie is their slow growth.

Drought

For the seedlings which attained temporary establishment, the principal immediate cause of death was drought. Fifty-seven per cent of all the species which germinated at either station during the two years perished during the last 10 days of June or in July. The decrease in numbers of individuals during both years showed even more clearly the destructive effect of hot, dry weather.

Soil moisture determinations were made from samples taken with a Briggs geotome as close to the seedlings as possible without disturbing their water

supply. The available water content was calculated by deducting the hygroscopic coefficient from the total water content. In the surface foot on the upland during the first year the water content was below this point (7.5) by July 11. In the surface 6 inches during the second year it was within 2 per cent of the limit of availability by July 1. The water content of the lowland soil at the level of the roots of the seedlings was always somewhat above the hygroscopic coefficient (10).

While the established vegetation protected the seedlings from the direct rays of the sun and the low humidity of the air above the plant cover, it deprived them of water. Its mass of underground parts, extending to within an inch of the soil surface, possessed many fine roots which absorbed at the same level as the seedling. As conditions of drought developed mature vegetation, transpiring strongly, depleted the upper soil of its moisture and continued to exist by reason of its deeper root system. By midsummer the surface foot of upland soil was too dry to maintain the life of seedlings.

Animals

Insects and other arthropods caused injury and complete destruction to large numbers of newly germinated seedlings from the time of their appearance to the middle of June. With drier weather and older plants, their activities were less conspicuous.

The fluctuating count of seedlings obtained in the spring of 1930, during successive weeks of observation, indicated high losses. The probability of damping-off fungi was not great on the open prairie, nor were remains of plants rotted at the base seen on the ground. The finely bitten appearance of the leaf tips and the diminished numbers of plants in a group suggested the depredations of insects and allied animals. A few beetles of small to medium size and rarely the moulted skin of a millepede were observed near the seedlings. Young grasshoppers and small, ground-living beetles constituted the most probable source of injuries due to animals.

Grasses found with the tops bitten off included *Agropyron smithii* and *Sporobolus asper* on upland, and *Bouteloua curtipendula* and *Spartina michauxiana* in lowland. Many forbs suffered partial destruction of their leaves. Practically complete loss of the seedlings of some species occurred early in the season, after conspicuous ravages had been made on the leaves. Replacement sometimes followed through additional germinations. Marked inroads were made each spring on the blazing stars. Many individuals of *Liatris punctata* and *L. scariosa* were continually pruned of their first typical leaf and reduced to the cotyledon stage.

The number of seedlings found was undoubtedly less than the total germination. Arthropods were decisive in preventing the development of many individuals. Young plants were destroyed or so weakened that they were unable to endure excessive shade or drought later in the season.

Slow Growth Due to Shade

When the food reserves in the seed or cotyledons have been exhausted, growth comes frequently to an apparent standstill. The period of greatest leaf formation and elongation for the majority of seedlings was found to follow immediately after germination. It seldom occurred later than the first week of June. The check to vigorous growth usually came before the season of drought. The cause appeared to be the presence of established vegetation. The permanent grasses had become so tall that the seedlings were too deeply shaded to make much food.

Clements and Weaver (1924) found that seeds planted in denuded quadrats produced 4 times as much establishment in prairie at Lincoln as seeds which were so planted that the seedlings grew in the shade of mature vegetation. Surface sowing resulted in competition at all periods, beginning with germination and continuing with development of seedlings which were soon undergoing shortage of both water and light. They prepared quadrats by destroying all vegetation present, so that only seedlings used the factors available for growth. Although the water relations were poorer because of the breaking of the cover of vegetation, the increase in light more than compensated for the decrease in moisture.

Light intensity in late May, at the surface of upland prairie burned the previous spring, was found, in the same study, to range from 2.5 to 26 per cent. In our experimental area, among the seedlings in July light commonly varied from 3 to 15 per cent in the upland and 2 to 10 per cent in the lowland. Readings as high as 62 per cent were taken among the upland seedlings, but the plants were small and wilted as the result of excessive transpiration and dry soil.

The late start of seedlings in spring was primarily responsible for their limited growth. Low temperature was the first retarding influence. While it was adequate in April for shoot production from perennating parts of mature plants, it was too low for rapid germination and seedling development. The average soil temperature in April (from records taken at Lincoln for 12 years) in the upper 3 inches of soil is 56° to 58°F. In May the averages are 10° to 12° higher. While an abrupt decrease in water content commonly occurs in late May, the first 3 weeks of the month present the optimum combination of habitat conditions for germination. Much the largest number of seedlings appeared during that period.

Where a group of seedlings consisted of closely crowded individuals, all died sometimes before the general water supply became critical. Eight specimens of *Gaura parviflora* in 1930 formed a small, uniform mass. From their germinations (April 25 to May 9) to their disappearance, almost no growth could be detected. On June 6 the clump was hardly more than a centimeter high, 5 plants were dead and the others wilted beyond recovery.

Inability to get enough water and nutrients in a much restricted area had so stunted them that they were unable to endure the first drying of the surface soil. In such instances death was due to competition among the seedlings.

Crowding of seedlings often resulted in the prompt death of some, the maintenance of suppressed existence by most and the elongation of a few far beyond the general level; *e.g., Salvia pitcheri.* The dominants survived the rest of their group by several weeks. They appeared to be little harmed by the stand of low, weak plants which surrounded them for a considerable time. Their greater stature and longer period of life emphasized the advantages of rapid growth as a factor in establishment.

The majority of individuals died within the space of a single week, probably during a critical day or two of extremely hot, dry weather. Such periods of drought are characteristic of prairie climate. Only once in a considerable number of years do they fail to occur. Well rooted plants alone can withstand them. Seedling growth in prairie is uniformly too slow to escape destruction.

CONCLUSIONS

Germination is retarded by the low temperatures of April. Insects destroy considerable numbers of very young plants. Seedlings present little foliage until new shoots of established vegetation are sufficiently enlarged to reduce greatly the light near ground level. As a result they are unable to form adequate leaf surface for photosynthesis. Their roots are starved for carbohydrates and deprived of water by the more efficient absorbing systems of mature, rapidly transpiring plants. Incapable of growing up into the light or of extending into the subsoil for water, seedlings usually lead a suppressed existence and perish in the first drought of summer.

Sufficient establishment obviously occurs in nature to maintain the prairie. The dominant plants of dry regions are highly specialized (Bews, 1929). In a climate poorly suited to the development of seedlings, a type of vegetation has been evolved which requires relatively little replacement. Long life of the individual and much vegetative reproduction are characteristic of grasslands. A few seedlings may become permanent each year, mainly in disturbed areas. Most of the establishment, however, probably occurs in natural prairie during the rare growing seasons of excessive moisture.

SURVIVAL OF SEEDLINGS DURING WINTER

The amount of winterkilling of seedlings found growing in the prairie in the latter part of October was studied during two separate years, 1929-1930 and 1931-1932. Seedlings were much more abundant in the former season than in the latter.

METHODS

Seedlings were located in unbroken prairie during the last ten days of October. Each individual or group of individuals growing closely together

was recorded and marked by a stake bearing a number. The middle of the following May, the stakes were relocated and the condition of the seedlings was observed.

CLIMATIC CONDITIONS

In 1929 the temperature during October was average with a maximum of 80°F. The minimum, 30°, did not occur until October 23. As killing frost was delayed until the sixth of November, and precipitation during October was very high, conditions favored germination and development of seedlings. The winter was, on the whole, severe. Snow covered the ground, however, much of the time until unusually high temperatures developed in February. In March the temperature was normal. The precipitation during both of these months was very low. In the absence of sufficient heat to stimulate growth, low precipitation does not appear to be injurious to prairie plants. Once they are well established, their endurance of drought during dormancy is great. Both temperature and precipitation were high in April, so that conditions were favorable for an early resumption of growth.

In 1931 the temperature in October was high, with a maximum of 91°F. on the tenth of the month and a minimum of 35°, not attained until the 31st. The precipitation was low. Germination on the uplands was prevented until very late in the month. Killing frost was recorded on November 1. The average temperature for November, however, was high (43.8°) and the precipitation was high, so that young seedlings probably made some growth. The winter temperatures were high except during January. An unusual amount of snow fell and the ground was covered most of the time. After a very warm March, April was cold. The precipitation during both months was well below the average. The May temperatures were high, while the precipitation continued low. Conditions in general were unfavorable for vigorous resumption of growth.

SPECIES

Erigeron ramosus and *Senecio plattensis* were the only forbs which regularly occurred in the fall in abundance. The seedlings of Erigeron were much the more numerous, as they often developed in thick clusters of plants. Senecio was represented by scattered individuals. Two species of Anemone were commonly present, *A. cylindrica* and *A. caroliniana* Walt. In the dry, hot autumn of 1931 Lithospermum germinated conspicuously. Thick cotyledons and occasionally a narrow leaf were present early in November. While seedlings of *Koeleria cristata* were abundant in the fall of 1929, only a few were found in 1931.

RESULTS

In 1929-1930 the survival of grasses appeared to be 100 per cent. About 135 seedlings of *Koeleria cristata* were located in the fall, forming low, dense groups. The number of plants present in the clumps in the middle of

the following May was larger. Whether or not some had died and been replaced by new germination, which was still occurring, could not be determined. Of a total of 44 forbs recorded, 32 were present in the spring, and with few exceptions were in a healthy condition. The spring inspection revealed the 3 plants of *Anemone cylindrica*, 4 of the 5 of *Aster multiflorus*, 11 of the 12 of *Erigeron ramosus*, the one specimen of *Psoralea esculenta* Pursh and all 13 plants of *Senecio plattensis*. A seedling of *Linum sulcatum* died, and 9 young plants which were too small to be identified. The total forb survival was 73 per cent.

In the fall of 1931 only 13 seedling grasses were found. They had such attenuated, wilted leaves that identification was difficult. Several plants of *Festuca octoflora* survived. They were, however, so nearly dead that it was practically certain that they would perish during the first hot days. Three specimens of *Poa pratensis* and 3 others which were not identified had disappeared by the middle of May. The survival of grasses was 54 per cent. Forbs were represented by 45 individuals. *Anemone caroliniana*, *Galium aparine*, Lepidium sp., and *Senecio plattensis*, each with a record of 2 seedlings, survived, though specimens of Lepidium were diseased. *Erigeron ramosus* lost 1 of 5 seedlings; Lactuca sp., 2 of 3; *Linum sulcatum*, 1 of 7; and Lithospermum sp., 2 of 16. Of 6 seedlings too small to be identified, 5 perished and the other was too little developed to be recognizable. The total forb survival was 75 per cent.

DISCUSSION

By far the largest proportion of deaths occurred among the seedlings which were too small to be identified. Very few such specimens survived and grew until they could be recognized. Most of the plants of Erigeron were in the 6 to 8 leaf stage. Anemone and Senecio often appeared to have lost one or 2 leaves when located in the fall. Lithospermum had very large cotyledons. The grasses which failed to survive entered the winter greatly weakened by drought. During both seasons of study, snow covered the ground throughout the greater part of the winter months. The thick layer of dead grass, however, affords much protection to young seedlings. Even in the absence of snow, they are not exposed to the direct action of the wind or to the most sudden changes in the temperature of the air.

Corroboration of the success of grasses in surviving the winter, if they have had good conditions for growth in the fall, was afforded by two species in the garden. Twelve plants of *Stipa spartea* and 20 of *Elymus canadensis*, all seedlings from seeds which were planted as soon as harvested, lived through the winter. By late December the leaves of Stipa had died back to a height of 2 cm. Those of Elymus were green for 5 cm. and apparently suffered little injury during the winter. Both species resumed active growth early in

spring. The winter survival of certain economic species which were seeded in the fall has been treated by Keim and Beadle (1927).

CONCLUSION

Vigorous fall seedlings of forbs which have developed to the third- or fourth-leaf stage or of grasses which have attained the second- or third-leaf stage show a survival through the winter of 80 to 100 per cent.

SUMMARY

Seeds of 42 species of plants of tall-grass prairie were tested for germination by plantings made *in soil*. The percentage of germination was ascertained and also the length of time after planting before the largest proportion of seedlings appeared.

The seeds were gathered when ripe from plants growing naturally in undisturbed prairie. A résumé is given of the environmental conditions under which they developed and to which germinating seeds and seedlings were subjected in nature.

Seeds of 10 species, planted 6 to 7 times during one year and in 8 successive months after another harvest, showed wide fluctuations in germinative power. It was, as a rule, very low at harvest time, highest in spring, often nearly as high early in the following autumn, but low in winter and midsummer. Frequently monthly variations were so great as to obscure the real viability, when germination was made the criterion. Hence, tests of germination should be made at the time of year most favorable to the development of the seeds. This is usually April and May.

Viability of seeds kept in dry storage at room temperatures was tested for 7 species of grasses and 4 of forbs. All except 2 grasses and 1 forb showed an almost complete loss of viability by the end of 5.5 years.

The seeds of 4 harvests were tested for 13 grasses and 18 forbs; those of 3 harvests for 2 other grasses and 9 other forbs. Several additional herbs were tested during 1 or 2 seasons. The percentage of germination varied considerably for any species from year to year and often from season to season. Germination of the seeds of most of the important grasses, when planted at a favorable time of year, ranged between 10 and 20 per cent; a few yielded over 40 per cent. The germination of the seeds of most forbs kept in dry storage was below 15 per cent, although in some species it was regularly at least 50 per cent.

The relative germination of seeds stored under different conditions was determined. Mature, air-dried seeds were stored out-of-doors but protected from precipitation and subjected to the freezing temperatures of winter. Similar seeds were stored indoors at living-room temperatures which were seldom below 50°F. In late winter, plantings from both lots were made in the greenhouse. Variability was observed from year to year in the state of

dormancy of the seeds at this season. For most species dormancy was broken for a larger proportion of the seeds which had been exposed to low temperatures. In addition many species were tested at other seasons. The time when a test was made was important for species in which it was difficult to break dormancy. Once the seeds were thoroughly ripened, a few species seemed to be almost as dependable as cultivated crops.

Stratification of seeds through the winter months resulted in marked improvement for many forbs and a few grasses. It produced the largest amount of increase in the germination of seeds of species which gave very low germination after dry storage either regularly or as the result of a poor growing season.

Most prairie species produced large numbers of seeds. Small but by no means negligible percentages of these were viable. The number of seedlings obtained varied greatly with the growing season preceding the harvest. Species producing seeds which gave germination of 50 to 80 per cent when grown in a relatively cool, moist summer were greatly handicapped in seed formation during seasons characterized by periods of drought. After such summers the same species sometimes produced seeds which gave a germination of less than 10 per cent.

The seeds of most prairie plants are subject to deep dormancy during the greater part of the year. Under conditions conducive to germination at seasons which are unfavorable for development they remain latent, no doubt with somewhat lowered vitality. Many develop subsequently if conditions again become suitable during the season which is normal to them for germination. The length of the latent period varied greatly with the season of planting. If this was favorable, most grass seeds germinated within 2 weeks and most forbs within 2 to 4 weeks. A few species of both grasses and forbs seldom produced many seedlings until the second month after planting.

Germination in the prairie was observed in spring and fall. There was relatively little as to both number of species and number of individuals. Comparatively few of the viable seeds which fell on the prairie germinated. Conditions were seldom favorable for a sufficient length of time for development of seedlings. Except for the few annual and short-lived plants, germination consisted of small numbers of widely scattered individuals which frequently failed to survive. Prairie sod brought into the greenhouse yielded relatively abundant germination in respect to both species and individuals.

Optimum water content for germination was found to be one-third to one-half saturation for seeds of species characteristic of uplands and one-half to two-thirds saturation for those of species of lowlands. Alternately high and low water content, with the resulting improvement in aeration, was satisfactory for seeds which germinated quickly.

The early life history of prairie plants has been studied in both controlled

and natural environments. A deeply penetrating root was developed rapidly, often with lateral branches before much of the shoot was exposed to water loss. This was a major adjustment to the prairie environment. Tillering in grasses began at the same time as the growth of the secondary root system. Organs for propagation and the accumulation of reserves of food were often developed very early.

Many of the long-lived species which do not set seed ordinarily until their second or third summer were found capable, under favorable conditions, of producing viable seed during the first year.

Establishment in the prairie sod was studied by measuring the development of seedlings from seeds planted in the spring. Because of heat and drought, no establishment was obtained from two years of planting. Although a few seedlings remained at the end of one summer, they winter-killed.

The survival of seedlings which had developed in autumn from seeds scattered by the wind was determined in the middle of the following spring. Vigorous seedlings which had attained the third- or fourth-leaf stage showed, during each of two successive seasons, a winter survival of 80 to 100 per cent.

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AN ECOLOGICAL STUDY OF THE FRESH-WATER SPONGES OF NORTHEASTERN WISCONSIN*

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* From the Limnological Laboratory of the Wisconsin Geological and Natural History Survey and the Thornton Junior College. Report No. 61.

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AN ECOLOGICAL STUDY OF THE FRESH-WATER SPONGES OF NORTHEASTERN WISCONSIN

HISTORICAL STATEMENT

Although fresh-water sponges have been the object of attention of students of freshwater biology for over half a century, and although the sporadic and erratic occurrence of sponges has been remarked by numerous workers, it is only recently that any definite attempts have been made to correlate sponge distribution with known environmental factors. One difficulty encountered by earlier workers has been the tendency to speak of "sponge distribution" as a single problem without specifying what species of sponge is under consideration. One would not attempt to discuss "bird habitats" without differentiating between woodpeckers and ostriches. It is not unlikely that as great a difference exists between the optimum habitats of two such sponges as *Ephydatia mülleri* (Lieberkühn) and *Ephydatia everetti* (Mills).

Potts (1887), records sponges from polluted waters "unfit for domestic use", but states their preference for pure waters. As to current he says "in my experience the finest specimens have always been found where they were subject to the most rapid currents. The lower side of large loose stones at the 'riffs', the rocks and foaming waters at the foot of a mill-dam fall, the timbers of a sluice-way, the casings of a turbine water wheel, or the walls of a 'tail race' beneath an old mill;—in all these places they have been found in great abundance and of very lusty growth." Shallow waters having a mud bottom are regarded as almost hopeless situations for sponges, whereas in water liable to be charged with heavy sedimentary material sponges may be sought on the lower sides of their bases of support or on perpendicular surfaces.

Modern limnological methods with their exact quantitative determinations were unknown in 1887. The paper by Potts is, nevertheless, a valuable contribution to the ecology of freshwater sponges for, under the discussion of each species which he collected himself, a description of a typical habitat is given. These descriptions, the work of an alert observer, are so vivid and accurate that the modern ecologist can all but check them against his chemically analyzed environment.

Stephens (1920) lists *Heteromyenia ryderi* Potts as a light-shunning form, and *H. repens* Potts, *Spongilla lacustris* (Linnaeus), and *Ephydatia fluviatilis* (auctorum), as limited by high calcium content. In her experience in Ireland she finds the most favorable sponge habitats to be rivers which drain lakes.

Smith (1921b) working in Michigan states, "There is abundant evidence that many favorable conditions for an abundant sponge fauna are present

in small bodies of water where there is not much wave action and where there is little or no silt. Enough current for circulation and renewal of the food supply is desirable, but only a few species thrive where the current is strong. Sloughs and channels which connect lakes are often favorable habitats."

Old (1932b), like Smith, fails to find the most sponges in the swiftest water and, contrary to the experience of Potts, records sponge colonies from the mud bottoms of ponds and from small sluggish streams. It is probable the "mud" from which Old collected sponges was largely organic detritus, whereas the "mud" found by Potts to be inimical to spouges was largely silt.

The author's interest in the *Spongillidae* was first aroused by Professor Frank Smith during her graduate work at the University of Illinois. Subsequently (in 1921) she again had the privilege of contact with Professor Smith at the University of Michigan Biological Station, and of accompanying him on several collecting trips. During such excursions conversation frequently turned to the variety of ecological problems furnished by sponge distribution, and unpublished observations, illustrative of the problems yet to be solved, were freely recounted by the elder scientist. He had found entirely different sponge faunas in two contiguous bodies of water. Sponges might be very numerous in one location while, in an apparently suitable location only a short distance away, not a specimen would be found. In one small bog, (Smith's Bog), which he had frequently visited, he had found a different sponge fauna at almost every visit; and the species most abundant at one time might be entirely lacking at a later date the same season, or at the same time the following season. While expressing the opinion that minute differences in the water, which a trained limnologist could detect, might account for these phenomena, he still refused to guess as to what these differences might be. When, in the summer of 1923, the author observed that Smith's Bog was not only subject to great variations in fauna, but that the quality of the water also underwent rapid fluctuations, changing from pH 4.3 to pH 6.8 within a period of three weeks, she promptly communicated this information to Professor Smith with the suggestion that, therein, might lie the solution to the sponge riddle. His reply was characteristic and generous, "I have enough Oligochaetes to keep me busy for some time, so am going to leave it to you to find out why sponges are distributed as they are; and shall be interested to know what you find out." While the author does not presume to interpret this remark as a "Cloak of Elijah", still this stimulating challenge may be regarded as remotely a cause of the present investigation.

Although this investigation was not begun until the spring of 1931, the desire to follow what appeared to be a good lead had smouldered for eight

years. It was, therefore, with great enthusiasm, that an invitation was received from Professor Juday to make use of the facilities of the Wisconsin Biological Survey while pursuing a study on the sponges of northeastern Wisconsin. Grateful acknowledgment is also made to Drs. Owen Nolf and Edward Schneberger for assistance in microphotography, and to the following students and members of the survey staff for cooperation in bringing in specimens and for assistance in handling boats and heavy apparatus: Dr. Edward Schneberger, Emerson McVey, Richard Wilson, Howard Field, and Hugo Baum.

It is doubtful whether any place in the world could be found better adapted to a study of the ecology of the *Spongillidae* than the Wisconsin State Biological Survey headquarters on Trout Lake. In Vilas County alone there are approximately eight hundred lakes and ponds, most of which have been mapped and are readily accessible. As a result of the extensive investigations of Drs. Birge and Juday with their corps of able assistants, one or more complete physical and chemical analyses has now been made of the waters of each of the larger and many of the smaller lakes. All of these data, unpublished as well as published, were most generously placed at the author's disposal; hence, to Drs. Juday and Birge, belongs the credit for the limnological data used in the following pages.

METHODS

In the smaller streams the sponges were collected by wading. In larger streams and lakes the collections were made from a boat. A garden rake was often used to bring submerged objects which might bear sponges within reach. The boats used for most of the collecting were a light 12 ft. duck boat and a pneumatic rubber boat. Sponges from depths of two or more meters collected from Crystal and Weber lakes were brought up by a Petersen dredge. Due to the weight of this apparatus it could not be used from either of the small portable boats. At the time of collection any characteristics of structure or growth form were recorded and the specimens were laid in wire screen trays to dry. When dry, a part of each sponge was mounted for identification and the remainder of the specimen placed in an envelope bearing the name, location, and date of collection.

Slides for rapid identification were prepared by boiling a fragment of the sponge on the slide in three or four drops of concentrated HNO_3, washing in alcohol, then xylol, and mounting in balsam. When better preparations were desired for more detailed study or microphotography, a fragment of the sponge was placed in a test tube with 10 to 15 cc. concentrated HNO_3 and allowed to stand three days. The test tube was then filled with distilled water, shaken thoroughly to separate the spicules from the organic debris, and allowed to settle for several hours. Most of the acid was then decanted

off and replaced by fresh water, and the process was repeated. After washing in this manner three times with water and once with 95% alcohol, fresh alcohol was added and the tube shaken to suspend the spicules. Drops of this alcohol containing spicules were then placed on cover glasses, dried by igniting the alcohol, and mounted on a slide with a drop of balsam.

The chemical methods used in analysis of the lake waters have already been described by Juday and Birge (1914, 1930, 1933). All analyses are expressed as mgms. per liter except transparency and conductivity. Transparency is expressed as the greatest depth in meters at which a standard Secchi disc is visible. No reading for transparency is given for several of the shallow lakes because the disc was plainly visible at the bottom. Conductivity is expressed as reciprocal megohms.

DATA

In all, 127 lakes and bogs, and 17 streams were examined, of which 103 lakes and 15 streams showed sponge faunas. 1389 sponges were examined microscopically in addition to a large number of specimens readily recognized without microscopic examination. The collections include 10 species, of which one, *Ephydatia everetti* (Mills), is a new report for the state and, so far as the author is able to determine, for the region west of the Alleghany mountains. This species, in addition to twelve species listed by Smith (1921) as occurring in Wisconsin, makes thirteen species of Spongillidae known to occur in the state. The species collected by the author were *Spongilla lacustris* (Linnaeus), *Spongilla fragilis* Leidy, *Spongilla ingloniformis* Potts, *Ephydatia mülleri* (Lieberkuhn), *Ephydatia everetti* (Mills), *Tubella pennsylvanica* Potts, *Heteromyenia argyrosperma* Potts, *Heteromyenia repens* Potts, *Heteromyenia ryderi* Potts, and *Carterius tubisperma* Mills. Of the species listed by Smith, three were not found by the author in the region studied, *Ephydatia fluviatilis* (auctorum), *E. crateriformis* (Potts), and *Carterius latitentus* Potts.

Table 1 gives the data for lakes and bogs, and Table 2 for the streams in which sponges were found. Lakes and streams in which no sponges were found have been omitted from the tables since in most cases failure to find sponges may have been due to inadequacy of collecting equipment. In two types of lakes only was the author convinced that failure to find sponges was due to their absence. This was the case in certain small senescent bogs choked with algae, and without sufficient exposure to wind to provide water movement; and in lakes of low transparency, regular shore line, and sand or gravel bottom, in which the effect of wave action extended deeper than light adequate for sponge growth. In addition to the chemical data presented in Table 1, the waters of the lakes from which sponges were collected have been analyzed for plankton, ammonia, organic N, NO_2, NO_3, total N, phospho-

rus, chlorine, and, in some, calcium. Since, however, not the slightest correlation appeared between any of these constituents and the occurrence of sponges, these analyses have been omitted. No chemical analysis is given for the streams, since the water is essentially similar to that of the lakes drained by them. Additional chemical data, as well as the description of the lakes studied, may be found in the works of Juday (1914), Juday and Birge (1930), or Birge and Juday (1934).

In Table 2, under the sponges collected, the fourth column is headed "*S. lacustris* (atypical). This heading is used to designate a type of *Spongilla lacustris* in which the dermal spicules, characteristically microspined, are either exceedingly slender and without microspines, or entirely wanting. This form was, at first, diagnosed as *Spongilla aspinosa* Potts 1880. As the work progressed, however, evidence accumulated which seemed to indicate that such Wisconsin specimens are not to be regarded as a distinct species but as variants of *S. lacustris*. Forms with attenuated skeletal elements were encountered in other species also, notably in *Tubella pennsylvanica*. In these cases, however, there was no sharp line of demarcation—such as presence or absence of spines—by means of which the variants might be certainly distinguished, so no attempt has been made to separate them in the table. A discussion of these forms is reserved until after the chemical data have been presented.

INTERPRETATION AND DISCUSSION OF DATA

An analysis of the data shown in Tables 1 and 2 shows that certain species are frequently associated whereas others have not been found together. The frequency of association of the various species is shown in Table 3 compiled from Tables 1 and 2.

Table 3 shows that of the 29 times *Ephydatia mülleri* was collected, it was found associated 27 times each with *Spongilla fragilis* and *S. lacustris*, and only 7 times with *Tubella pennsylvanica*. *Spongilla fragilis* was taken 55 times, of which it was associated with *Ephydatia mülleri* 27 times, with *Spongilla lacustris* 45 times, with *Tubella pennsylvanica* 16 times, and occurred alone 8 times. At the other extreme, the atypical form of *Spongilla lacustris*, which was collected from 20 lakes, occurred 11 times with *Tubella pennsylvanica*, once with *Spongilla ingloviformis*, 10 times with *Ephydatia everetti*, and 5 times alone, but was never found coexistent with *Ephydatia mülleri*, *Spongilla fragilis*, or the typical forms of *S. lacustris*. Two distinct and mutually exclusive association groups thus appear among the sponges, one consisting of *Ephydatia mülleri* and *Spongilla fragilis*, the other of *Ephydatia everetti* and the atypical *Spongilla lacustris*. Such a distribution indicates the probability that certain environmental factors, necessary for one group of sponges, are prohibitive to another.

TABLE 1

Lakes	Disc	O_2	Free CO_2	Fixed CO_2	pH	SiO_2	Conductivity	Residue	Color	E. mulleri	S. fragilis	S. lacustris	S. lacustris (atyp.)	T. pennsylvanica	S. ingloviformis	E. everetti	H. argyrosperma	H. repens	H. ryderi	C. tubisperma
Allequash	1.5	9.4	—1.75	16.5	8.6	5.2	61	56.8	36	..	*	*	..	..	..	..	..	..	..	..
Anna	1.3	.....	2.0	2.0	6.5	0.45	19	41.8	74	..	*	*	..	*	..	..	..	..	..	..
Anne	2.3	9.7	1.5	2.5	5.1	0.3	12.2	18.2	24	..	..	..	*	..	..	..	..	..	..	..
Arborvitae, Big	.....	10	—1.5	21.2	8.4	3.3	88	70.5	24	*	*	*	..	..	..	..	..	..	..	..
Bass (by N. Pickerel)	.....	8.0	2.0	2.5	5.3	0.2	13.5	....	6	..	..	..	..	..	*	*	..	..	..	..
Bear	3.0	9.4	0.5	3.5	5.9	0.8	13	20.7	28	..	..	*	..	*	..	*	..	..	..	..
Big	2.0	8.4	—1.2	20.3	8.3	4.0	96	70.2	25	*	*	*	..	..	..	..	..	..	..	..
Bog (on Black Oak Road)	.....	8.1	7.0	22.0	7.3	19	87	71.5	33	..	*	*	..	..	..	..	..	..	..	..
Bog (by Little Dear Lake)	.....	.....	......	......	.....	......	.....	.....	.....	..	..	..	*	*	..	..	..	..	..	..
Bog (by Buck Lake)	.....	.....	......	......	.....	......	.....	.....	.....	..	..	*	..	*	..	..	..	..	*	..
Boulder	1.9	7.7	3.0	12.1	7.2	7.0	56	50.4	64	*	*	*	..	..	..	..	..	..	..	..
Bug	1.8	7.1	2.5	4.0	6.5	1.0	17.7	27.9	53	..	*	..	..	*	..	..	..	*	..	..
Camp	5.3	6.9	1.5	0.5	5.8	Trace	11.9	11.1	13	..	..	..	*	*	..	*	..	..	..	..
Carlin	7.5	7.9	1.0	1.4	6.4	Trace	12.5	15.2	8	..	..	..	*	..	..	..	..	..	..	..
Clear (Oneida Co.)	8.1	8.6	1.0	4.5	6.1	Trace	17	17.1	0	..	..	..	*	..	..	..	..	..	..	..
Crane	4.5	8.1	1.0	2.5	5.6	0.15	14.9	16.3	15	..	..	..	*	..	..	*	..	..	..	..
Crawling Stone	3.0	7.2	1.5	11.3	7.4	1.2	58	36.1	0	..	*	*	..	..	..	..	..	..	..	..
Crystal	12.7	8.2	1.0	0.5	5.1	Trace	11	29.4	0	..	..	..	..	..	..	*	..	..	..	..
Curtis	4.5	.....	2.3	0.6	6.2	Trace	13	12.5	8	..	..	..	..	..	*	*	..	..	..	..
Dam	0.9	6.1	2.0	6.0	6.7	5.0	33	52.9	94	*	*	*	..	..	..	..	..	..	..	..
Elizabeth	.....	.....	2.3	3.0	5.0	0.7	11	15.8	6	..	..	..	..	*	*	*	..	..	..	..
Ellerson, Middle	5.0	8.5	1.2	1.3	6.4	Trace	11.1	19.1	0	..	..	..	..	*	..	..	..	..	..	..
Ellerson, West	.....	10.5	4.3	22.1	8.5	8.0	95	77.6	26	..	*	*	..	..	..	..	..	..	..	..
Flato	.....	9.4	1.7	4.5	6.1	Trace	7.5	16.5	16	..	..	..	..	..	..	*	..	..	..	..
George (N. of Winchester)	3.0	8.8	1.0	4.0	6.2	0.3	20	33.5	43	..	*	*	..	*	..	..	..	..	..	..
George (Black Oak Road)	.....	8.4	3.0	9.0	6.7	1.0	26	....	14	..	..	*	..	..	..	..	..	..	..	..
Grassy	.....	6.5	6.2	10.2	6.8	4.0	38	44.6	53	*	*	*	..	*	..	..	*	*	..	..
Grassy (by Wishau)	.....	6.7	2.0	3.5	6.2	0.5	12	19.7	8	..	..	..	..	*	..	..	..	..	..	..
Gresham, Upper	8.2	6.7	3.4	16.9	7.0	5.6	74	61.0	12	..	*	..	..	..	..	..	..	..	..	..
Gunlock	1.8	8.1	1.2	16.5	7.4	4.0	68	57.3	32	..	*	..	..	..	..	..	..	..	..	..
Harvey	0.75	8.1	0.5	6.9	7.6	3.6	35	79	202	..	*	*	..	*	..	..	*	..	..	..
Helen (by Winchester)	1.6	6.8	4.0	2.5	6.0	0.4	18.5	39.2	97	..	..	*	..	*	*	..	..	..	..	..
Helmet	0.8	.....	5.0	2.0	5.6	4.4	19	71	268	..	..	..	..	*	*	..	..	..	..	..
High	1.9	10.2	0.8	23.0	7.4	6.0	85	66.9	22	..	*	*	..	..	..	..	..	..	..	..
Hurrah	5.0	8.8	2.0	3.0	6.4	0.3	18	20.8	15	..	..	*	..	*	..	..	..	..	..	..
Irving	.....	8.3	2.0	13.0	7.2	7.2	56	56.8	32	..	*	*	..	..	..	..	..	..	..	..
Joyce	4.0	7.3	1.7	0.7	6.0	Trace	9	15.3	11	..	..	..	*	..	..	*	..	..	..	..
Katinka	6.0	7.8	0.5	1.7	6.1	Trace	12.9	17.1	10	..	..	..	*	..	..	..	..	..	..	..
Larry	6.5	9.6	1.2	0.7	5.1	Trace	11	18.6	8	..	..	..	*	*	..	*	..	..	..	..
Little Arborvitae	2.6	7.8	2.0	21.5	7.4	4.0	87	73.5	26	*	*	*	..	..	..	..	..	..	..	..
Little Crooked	2.2	7.0	2.0	23.0	7.6	3.0	92	67.1	22	..	..	*	..	..	..	..	..	..	..	..
Little Horsehead	.....	.....	......	11.1	7.0	2.0	52	58.1	58	*	*	*	..	*	..	..	..	..	..	..
Little John, Jr.	2.3	7.3	2.0	0.55	5.7	Trace	9.5	19.1	16	..	..	..	..	*	*	..	..	..	..	..
Little Long	1.5	7.6	2.2	6.5	7.1	2.4	28	50.8	68	..	*	*	..	*	..	..	..	..	..	..
Little Maimie	3.0	8.4	2.0	1.25	5.4	0.25	9.8	20	16	..	..	..	..	*	*	*	..	..	..	..
Little Muskelunge	1.9	8.2	0.6	18.5	8.1	6.6	78	61.9	43	..	*	..	..	..	..	..	..	..	..	..
Little Papoose	2.3	8.1	1.0	20.0	7.0	2.5	78	68.6	49	*	*	*	..	..	..	..	..	*	..	..
Little Pickerel	1.1	7.6	4.5	8.0	6.8	12.0	28	70.6	108	..	..	*	..	*	..	..	..	..	..	..
Little Rice	.....	10.3	1.6	8.4	7.2	7.0	45	45.3	43	*	*	*	..	*	..	..	*	..	..	..
Little Rock	.....	5.6	2.0	0.7	5.7	Trace	11	14.9	8	..	..	..	*	*	..	*	..	..	..	..
Little Rudolph	3.5	7.5	1.6	0.8	5.9	Trace	8.2	13.8	5	..	..	..	*	*	..	..	..	..	..	..

TABLE 1 *(Continued)*

Lakes	Disc	O_2	Free CO_2	Fixed CO_2	pH	SiO_2	Conductivity	Residue	Color	E. mulleri	S. fragilis	S. lacustris	S. lacustris (atyp.)	T. pennsylvanica	S. ingloviformis	E. everetti	H. argyrosperma	H. repens	H. ryderi	C. tubisperma
Little St. Germaine	1.7	10.7	—0.5	13.4	7.3	0.7	58	42.7	14	*	*									
Long (by Crystal)	4.4	7.6	1.5	1.5	5.5	0.35	9.5	14.2	20				*			*				
Louise	.....	6.8	3.0	1.7	5.8	0.3	10	16.5	8					*	*	*				
Lucy	2.3	8.3	0.7	2.2	8.3	1.6	17	28.5	32			*		*						
Lynx	.....	6.5	2.2	1.0	6.1	Trace	16.5	24.9	30			*								
Malby	.....	.....	1.5	0.9	6.0	0.25	12.5	13.8	15					*	*	*				
Mann	.....	8.8	—2.7	25.55	8.0	6.0	105	93.2	20		*									
Mary (by Winchester)	1.7	6.0	5.5	3.0	5.4	1.0	19.5	51.3	132			*		*	*					
Mary (by Papoose)	3.5	8.8	2.5	3.0	5.7	0.25	11.5	15.4	14				*							
Midge	3.2	6.9	1.5	2.5	5.4	Trace	11.0	20.5	35				*	*						
Mud	3.0	7.2	2.0	6.0	6.3	0.3	17	25.8	45			*								
Muskelunge	3.5	8.4	1.2	10.5	7.4	0.3	42	31.2	5			*					*			
Muskelunge (by Pickerel)	0.5	6.6	......	14.9	6.9	10.0	62	86.4	105		*									
Nebish	7.3	8.2	1.5	4.5	6.6	0.4	19	21.9	5			*				*				
Nellie	1.3	5.0	1.2	0.7	6.5	0.5	10.8	26.5	118					*						
Nixon	.....	8.5	1.0	13.0	7.1	5.0	47	66.6	118	*	*	*								
No-see-em	1.0	7.3	5.0	4.0	5.2	0.5	19	50.0	148			*		*	*					
Oswego	2.7	9.0	2.5	2.5	6.2	0.4	13	17.9	18				*	*		*				
Ox bow	1.1	7.2	1.7	9.0	6.9	1.5	34	61.2	97		*	*		*						
Papoose	2.8	8.0	1.2	23.5	7.7	1.6	88	74.0	18	*	*	*								
Pardee	1.1	.....	......	14.0	7.6	2.5	65	65.9	68		*									
Perch (Ruth)	5.5	6.4	1.4	0.5	5.9	Trace	9.5	14.3	20				*	*	*	*				
Pickerel	0.9	7.7	......	15.5	7.1	5.5	64	74.3	53			*		*			*			
Plum	4.7	8.0	0.8	16.4	8.0	4.6	73	58.0	16			*								
Presque Isle	3.8	8.8	—1.0	27.5	8.3	0.8	101	71.7	14		*	*								
Puddle	.....	4.4	2.5	6.5	6.6	2.0	54	50.0	43	*	*	*		*						
Rice, Big	1.7	.....	......	16.3	7.3	5.3	70	54.2	40	*		*								
Rice (by Plum)	.....	4.0	13.9	17.1	6.6	8.2	70	65.4	88	*	*	*								*
Rock	2.1	8.5	0.5	10.0	7.2	0.6	37	43.7	32			*								
Rose	1.1	8.4	1.8	2.7	6.7	2.5	25	53.9	128			*		*						
Rudolph	1.8	7.4	1.5	2.5	5.6	0.3	17	34.0	55			*								
Shishebogoma	3.0	8.6	1.3	14.7	7.3	4.0	67	54.0	16		*			*						
Street	5.0	7.2	1.8	0.7	5.9	Trace	10	14.5	0							*				
Stella	.....	8.7	1.7	15.3	7.3	8.8	66	66.8	101		*	*		*						
Sunday	4.0	..	1.3	1.6	6.7	0.3	13	18.9	0				*	*						
Tadpole	0.9	7.8	1.0	2.7	5.6	0.9	20	57.3	101			*		*	*					
Tamarack	1.7	6.3	3.0	13.4	7.1	2.1	67	75	101	*	*	*		*				*		
Trilby (1931)	.....	8.0	1.5	1.0	7.0	0	13	14.2	14				*	*						
Trilby (1934)	.....	.....	1.4	2.1	7.5	0.5	14	18.2	12			*		*	*	*				
Trout	5.0	8.5	2.0	18.5	7.5	7.3	75	59.5	6	*	*	*								
Turtle	2.2	4.7	1.5	7.0	7.4	2.0	56	67.9	50	*	*	*								
Twin, N	.....	.....	4.0	11.0	7.2	13.0	31	....	.....			*								
Vieux Desert	1.3	8.0	1.1	16.4	7.2	2.4	64	58.0	32		*									
Weber	9.3	8.3	1.0	2.0	5.8	0.1	9.5	12.2	0							*				
White Birch	3.5	8.8	0.7	13.2	7.6	4.5	56	51.2	60		*	*								
White Sand	3.7	8.4	1.4	15.9	7.7	5.4	57	51.1	16	*	*	*								
Wild Cat	2.3	7.9	0.3	33.2	8.3	3.6	118	88.7	26		*	*								
Wishau	.....	7.4	1.5	2.0	6.0	0.3	10	15.0	0				*	*		*				
Wolf	2.2	8.5	4.5	26.0	8.0	2.6	90	87.2	32		*									
Walker	2.2	8.4	2.0	1.5	5.8	0.2	8	22.4	35				*			*				
Yolanda	1.0	6.8	4.2	2.5	5.5	0.4	15.2	43.3	132			*		*	*					

TABLE 2

Stream	Character	E. mulleri	S. fragilis	S. lacustris	T. pennsylvanica	H. argyrosperma	H. repens	H. ryderi	C. tubisperma
Allequash inlet	Spring fed, bog margin		*	*					
Allequash outlet	Gentle current, bog margin	*		*					
Big Lake outlet	Wide, shallow, over gravel bottom	*	*	*					
Little Horsehead outlet	Rapids, rock bottom	*		*		*			*
Little St. Germaine outlet.	Ponded by a dam, little current	*	*						
Manitowish River									
Below Little Rice Lake	Sluggish, boggy		*	*	*				
Below Boulder Lake	Rapids, rock bottom	*	*	*					
Mann Lake outlet	Riffel, rock bottom	*	*	*			*		*
Mud Creek	Ponded by dam, little current	*	*	*	*	*			
Plum Lake outlet	Shallow, gentle current over gravel bottom			*					
Presque Isle outlet	Both ponds and riffels		*	*					
Rice Creek	Ponded by dam	*	*	*	*	*			
Trout Lake inlet	Spring fed, gentle current, bog margin	*	*	*				*	
Trout Lake outlet	Current over gravel bottom, wooded margin		*	*					
Turtle Lake outlet	Current over gravel and pebbles		*	*		*			
Wisconsin River									
above dam	Sluggish with much wood debris	*	*	*		*			
Otter Rapids	Strong current over rock bottom	*	*						

TABLE 3. Showing the frequency of association of the species of *Spongillidae* collected

No. of times collected	Species	E. mulleri	S. fragilis	S. lacustris	T. pennsylvanica	S. ingloviformis	E. everetti	S. lacustris (atypical)	H. argyrosperms	H. repens	H. ryderi	C. tubisperms
29	E. mulleri	..	27	27	7	..	..	..	6	4	1	3
55	S. fragilis	27	8	45	16	..	..	..	6	4	1	2
70	S. lacustris	27	45	11	27	12	2	..	10	5	2	3
49	T. pennsylvanica	7	16	27	3	14	11	11	6	3	1	..
15	S. ingloviformis	..	..	6	14	..	7	1	..	..	..	..
22	E. everetti	..	..	2	11	7	4	10	..	..	..	..
20	S. lacustris (atypical)	..	..	..	11	1	10	5	..	..	..	..
10	H. argyrosperms	6	6	10	6	..	..	..	..	1	..	1
5	H. repens	4	4	5	..	..	..	..	..	..	..	1
2	H. ryderi	1	1	2	..	..	..	..	..	..	..	1
3	C. tubisperma	3	2	3	..	..	..	..	.	1	1	..

The typical form of *Spongilla lacustris*, although overlapping slightly the ecological range of *Ephydatia everetti*, is never found coexistent with the atypical form, and shows a decided preference for the conditions favoring *Ephydatia mülleri* and *Spongilla fragilis*. *Tubella pennsylvanica* is the only species found coexisting with all of the other forms, a fact which suggests

that its distribution is controlled by a still different complex of environmental factors than those which separate *Ephydatia mülleri* and its associates from *Ephydatia everetti* and its.

Spongilla ingloviformis presents still another set of problems. Although never taken with *Ephydatia mülleri* or *Spongilla fragilis*, it can not be classed with *Ephydatia everetti* and the atypical forms of *Spongilla lacustris* because of its low association with the latter and only moderately frequent association with the former. On the other hand, its association with *Tubella pennsylvanica* is very high, 13 times out of 15.

The Heteromyenias are associated with *Spongilla lacustris*, and usually also with *Ephydatia mülleri* and *Spongilla fragilis*, although the low frequency of their collection indicates the requirement of some condition not demanded by the more common forms.

In addition to the question of factors controlling the presence or absence of the various species, there is also the problem of possible factors producing the marked structural variations already mentioned as occurring in *Tubella pennsylvanica*. Since its first description, *Tubella pennsylvanica* has been known as a highly variable species. It was, then, not surprising that the author should find forms so divergent that, were there no intergradents, they might readily be accepted as distinct species. The "typical", or usually described and figured form of *T. pennsylvanica* is characterized by gemmules thickly studded with inequaebirotulate spicules arranged at right angles to the gemmule wall, and having rotules with entire margin (Fig. 18). The shaft of these birotules is enlarged at the end adjoining the larger, or proximal, rotule, and the entire structure is surrounded by a crust of thickly spined acerates. The most common variation from this "typical" form was a sponge having gemmule walls containing very few spicules. The few inaequibirotulates present consisted of a minute knob shaped distal rotule scarcely larger than the shaft, and a proportionately long and exceedingly slender shaft (Figures 17, 19, 20 and 21). In some cases the proximal rotule was apparently not silicified, its position being indicated merely by an indistinct outline in the structure of the organic matrix. In other specimens many of the scattered inaequibirotulates were not standing upright but lay on their sides. The acerate spicules surrounding the gemmule were also sparse, so that the gemmule wall had the appearance of being composed, largely, of a hyaline leathery material like the gemmule walls of *Spongilla lacustris*. The vegetative parts of such sponges were frequently well developed. The skeletal spicules were extremely long and slender, (Fig. 22), and sometimes curved, so that the sponge skeleton had the appearance of a mass of tangled barb wire. Specimens of this kind were encountered in the bog near Little Deer Lake, and in the following lakes: Camp, Middle Ellerson, Grassy (Near Wishau), Larry, Little John Junior, Little Maimie, Little Rock, Little Rudolph, Louise, Malby,

Midge, Oswego, Perch, Sunday, Trilby (1932), and Wishau. Specimens somewhat less atypical, but regarded as intermediate in skeletal development, appeared in lakes Elizabeth, Hurrah, Nellie, No-see-em, and Trilby (1934): whereas, even among specimens readily recognized as *Tubella pennsylvanica,* there appeared great variations in the degree of development of the gemmule spicules. It was, furthermore, observed that wherever these unusual forms appeared, all of the specimens from that lake showed the same variation to approximately the same degree, suggesting that these variations were due to some environmental condition unfavorable to skeletal development. And, finally, it will be observed that the lakes in which these atypical Tubellas occurred include all of the lakes in which this species is associated with the atypical forms of *Spongilla lacustris,* 9 of the 11 lakes in which it is associated with *Ephydatia everetti,* and 4 lakes in which it is the only species present. This being the case, it appears probable that the same environmental factors which so sharply separate the atypical forms of *Spongilla lacustris* from *Ephydatia mülleri* and its associates may also produce skeleton-poor Tubellas.

In analyzing environmental data one must, then, except at least three different sets of factors affecting the distribution of the sponges: (1) factors favoring *Ephydatia mülleri, Spongilla fragilis,* and *Spongilla lacustris* but inimical to *Ephydatia everetti;* (2) factors inimical to *Ephydatia mülleri* and *Spongilla fragilis,* tolerated to a slight degree by *Spongilla lacustris,* but favorable to *Ephydatia everetti* and the atypical forms of *Spongilla lacustris* and probably also affecting the spicule development in *Tubella pennsylvanica;* and (3) factors favoring *Spongilla igloviformis* and probably also *Tubella pennsylvanica,* but either inimical or indifferent to the remaining forms.

In seeking an explanation of animal distribution in terms of the physical and chemical environment there are three ways in which data are frequently handled: (1) the entire range of each factor known to be tolerated by each species may be compared, (2) the frequency of each species in each type of environment may be compared, and (3) the optimum environment for each species may be compared. In the following pages all three methods are employed. Except in the discussion of current, the data on lakes and bogs (Table 1) only are used.

Figure 1 represents diagramatically the range of environmental conditions from which each species was collected. The lines represent the entire range of conditions found in the 127 lakes studied whereas the black part of each line represents the range through which the species in question was found. Tables 4 to 13 inclusive give the frequency of occurrence of each species in the various concentrations of environmental factors under consideration, and Table 14 shows the range of conditions under which each species was found in greatest luxuriance and abundance.

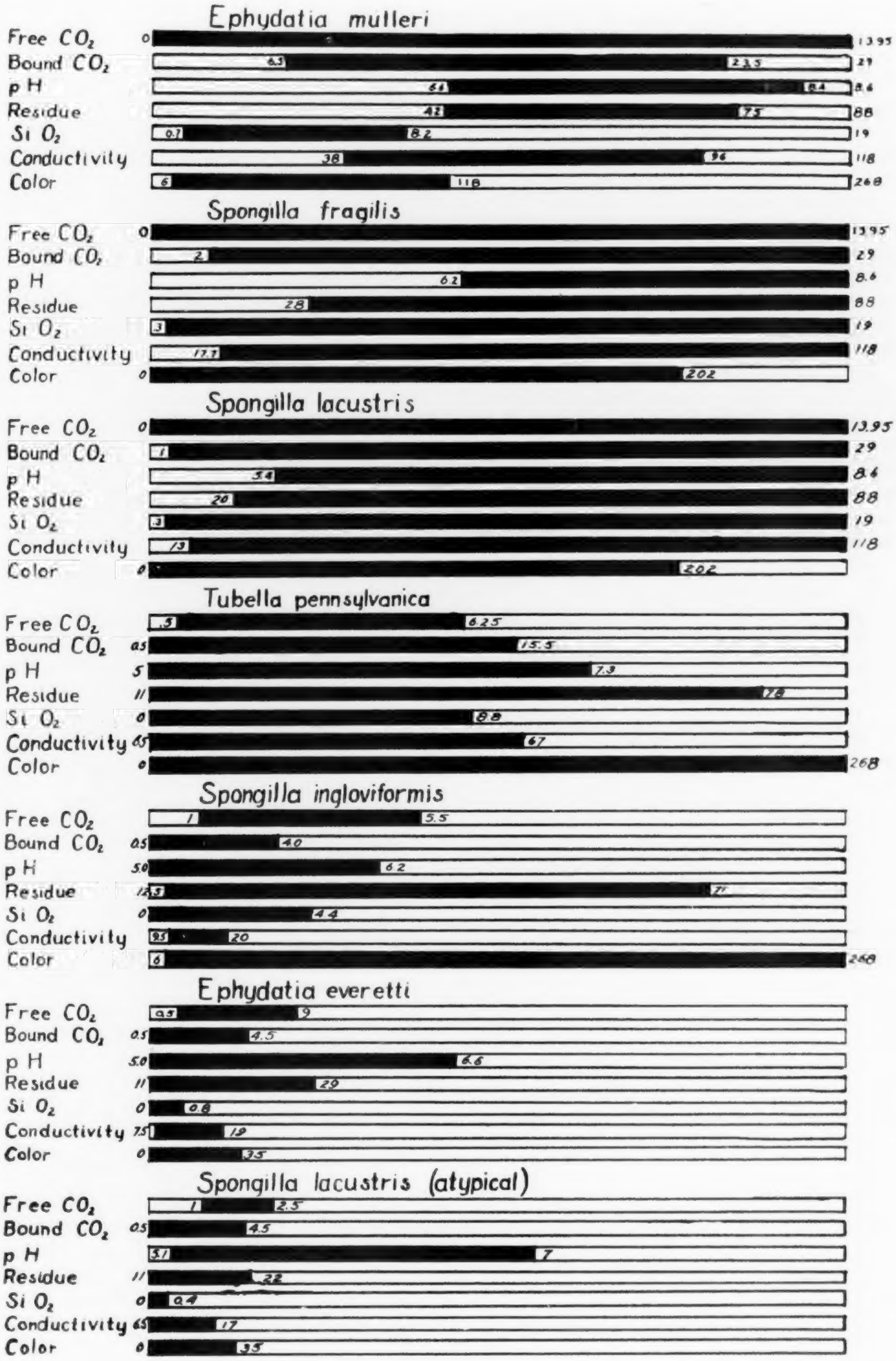

FIGURE 1. Diagram showing the distribution of fresh-water sponges with reference to the physical and chemical factors of the water. The entire line represents the range of each condition found in the series of lakes studied. The black part of the line represents the range from which the species of sponge under consideration was collected.

DISTRIBUTION OF SPECIES WITH RESPECT TO THE PHYSICAL AND CHEMICAL ENVIRONMENT

TRANSPARENCY OF THE WATER

TABLE 4. Distribution of sponges with reference to the transparency of the water

Sponge	Transparency as meters visibility of Secchi's disc.							
	0-1	1.1-1.5	1.6-2	2.1-3	3.1-4	4.1-5	5.1-6	Over 6
E. mülleri	1	.	5	4	1	1	.	.
S. fragilis	3	6	8	10	4	1	.	.
S. lacustris	6	6	9	12	4	3	.	1
T. pennsylvanica	6	6	4	7	3	1	3	1
S. ingloviformis	4	.	2	2	.	1	1	.
E. everetti	.	.	.	4	1	4	3	4
S. lacustris (atypical)	.	.	.	3	5	2	3	3
Lakes	10	9	14	24	11	13	5	8

In considering the relation of sponge distribution to the transparency of the water, the data in the above table show little except the absence of *Ephydatia everetti* and the atypical *Spongilla lacustris* from the less transparent waters. Transparency of the water then appears to be of little importance in determining whether any species except the two mentioned shall occur in any given lake or not. It does, however, appear from field observations, that transparency may be of considerable importance in determining the depth, and location with respect to shade, at which any species may be found. Thus, in waters of low transparency, like those of Harvey, Little Pickerel, or Rose, *Spongilla lacustris* was found, frequently in great abundance, growing from a depth of a few centimeters to a half meter up to the surface in unshaded situations; whereas in more transparent waters, like those of Little Papoose, Nebish, or White Sand, the same species was collected near the surface in partially shaded situations, as on the submerged stems and roots of Chamaedaphne shrubs, or, at a depth of 1 to 1.5 meters in less shaded situations. The relation of *Ephydatia mülleri* to light appears to be essentially the same as for *Spongilla lacustris*. *S. fragilis*, on account of its ability to grow well on the under side of the substrate, is more frequently found under logs in unshaded transparent waters.

Ephydatia everetti and the atypical *Spongilla lacustris* are apparently light positive forms, occurring in exposed situations in the most transparent waters at depths of from 0.2 meters to over 3 meters. In Weber and Bass lakes *Ephydatia everetti* reached its maximum abundance at a depth of 1.5 meters and was still common at 2 meters. In Weber it was still found at 3 meters. In Louise, great festoons of this form were visible at a depth of 2.5 to 3 meters, in Crystal it was collected at 3.5 meters, while in Larry

and Joyce both *Ephydatia everetti* and the atypical *Spongilla lacustris* persisted to a depth of about 3.5 meters. The record for Crystal Lake, 3.5 meters, is the greatest accurately measured depth from which the author has obtained any sponge.

Tubella pennsylvanica and *Spongilla ingloviformis* are both found in light of low intensity. Although collected from some of the most transparent waters it was observed that, in such situations, both species invariably occurred in the under side of the support or actually embedded in the organic deposits of the bottom. Thus, in Little John Junior, *Tubella pennsylvanica* is recorded as found on the under side of wood partially covered by the decomposing organic matter of the bottom, and in Camp Lake the same sponge was found on the embedded parts of sticks pulled from a bottom which emitted gas bubbles when the sticks were removed. *Spongilla ingloviformis* was taken from the under sides of logs and boards resting on the bottom in Curtis and Little Mamie lakes, while in Malby it was found in abundance in similar locations, and also on both the upper and lower sides of logs brought up from a depth of 1.5 meters but entirely concealed from the surface by a rank growth of filamentous algae which rose, in billows, almost to the surface. In lakes of low transparency, like Helmet, Tadpole, and Yolanda, the same two species occurred on the upper as well as the lower surfaces of their supports and only a few centimeters below the surface. One curious fact is that even these light negative forms were never collected below the limit of light penetration as judged by the visibility of Secchi's disc, and that the sponge collected at the greatest depth was the light positive form, *Ephydatia everetti*.

COLOR AND ORGANIC CONTENT OF THE WATER

Color of water, although often closely associated with transparency, is, nevertheless, a distinct factor. A decreased transparency may result from either color or suspended matter, whereas color is the result of substances, usually organic, in solution.

Birge and Juday (1932) have shown that whereas as much as 30 to 39% of the solar energy with the sun at zenith is transmitted to a depth of one meter in lakes such as Crystal, Weber, or Clear, that in Turtle Lake, with a color of 68, only 6.4% reaches a corresponding depth, while in still darker lakes, Little Pickerel and Helmet with colors of 108 and 260, the transmission of solar energy at a depth of 1 meter drops to 3.5% and 1% respectively. Furthermore there is not only a decrease in the total amount of energy transmitted, but the various wave lengths are absorbed at different rates depending upon the color of the water, so that the qualitative effect upon the light transmitted by a highly colored water may be very different from that of a colorless but turbid water of the same transparency.

That the effect of color on sponge life is not simply a matter of decreased

light is evident, since waters of high color were usually rich in sponges, whereas waters of little or no color, but of low transparency, even though the turbidity was due to plankton, were invariably poor to lacking in sponge life.

As the color of water is usually due to dissolved organic matter, it is roughly proportional to organic content, and so indicates the amount of nutriment available to such animals as sponges. Because of this correlation the full data for organic content have not been included in Table 1. Table 5 shows, however, that the effect upon sponge distribution of color and of total organic matter, is practically the same. With an increase in color, then, the food available to sponges is usually increased, whereas the light available is qualitatively altered and quantitatively decreased.

TABLE 5. Distribution of Sponges with reference to Color and Total Organic Content of the water

Sponge	Degree of color of water								
	0-10	11-20	21-30	31-40	14-50	51-75	76-100	101-150	over150
E. mülleri	1	3	3	1	4	3	2	2	.
S. fragilis	3	7	5	6	7	8	3	4	1
S. lacustris	5	6	9	7	5	8	4	9	1
T. pennsylvanica	9	9	1	2	3	6	2	9	2
S. ingloviformis	4	4	.	.	.	.	1	4	1
E. everetti	11	9	1	1	.	.	.	.	.
S. lacustris (atypical)	8	7	1	2	.	.	.	.	.
Lakes	36	23	14	14	6	13	4	11	2

Sponge	Total Organic content as mgms. per liter						
	3.5-5	5.1-7	7.1-10	10.1-15	15.1-25	25.1-40	Over50
E. mülleri	.	.	2	6	8	3	.
S. fragilis	.	2	2	14	16	8	1
S. lacustris	.	3	4	15	16	13	1
T. pennsylvanica	3	5	7	6	8	11	2
S. ingloviformis	3	1	2	2	.	4	1
E. everetti	6	4	9	3	.	.	.
S. lacustris (atypical)	2	4	9	3	1	.	.
Lakes	6	6	19	24	25	14	2

A comparison of Table 5 and Fig. 1, shows that, except in case of *Ephydatia everetti* and the atypical *Spongilla lacustris,* color, or the corresponding organic content, have no apparent effect in limiting the distribution of these sponges studied. The absence of *Ephydatia mülleri* from 8 lakes having colors above 118, and from 10 lakes having colors below 6, can not be regarded as more than suggestive, since, of these lakes, only two, Crawling Stone and Harvey, were not also lower in either or both conductivity and silica than any water from which this species was taken.

Although apparently not important in determining the presence or absence

of any species, except possibly *Ephydatia everetti*, color (or the accompanying high organic content) is of primary importance in determining the abundance of most species of sponges in waters otherwise suitable, as shown by examination of Table 15, in which the optimum habitats are compared. Here, it will be observed that three species, *Ephydatia mülleri, Spongilla fragilis*, and *S. lacustris*, attain their finest development in waters relatively high in color (less than 30% of the lakes have colors of 40 or above), whereas the luxuriance of *Tubella pennsylvanica* appears to be almost a function of the color of the water. *Spongilla igloviformis*, although attaining numerical abundance and extensive growth in two clear water lakes—Elizabeth and Malby—where it developed in contact with bottoms rich in organic detritus, still showed by far the most luxuriant development in the darker waters, and reached its climax in the lake of highest color—Helmet. In the clear lake waters representatives of this species appeared as delicate uniform incrustations, bright green in color, and 5 to 7 mm. thick. In the darker waters they became heavily incrusting to massive, rugose, vivid to dark green, and up to 25 mm. in thickness, (Figs. 8 and 9).

Although Helmet Lake, which supports an almost unbelievable abundance of *Spongilla igloviformis* and *Tubella pennsylvanica*, provides scarcely any suitable substrate in the upper half meter of water which is not already occupied by one or both of these species—one frequently growing right over the other—still it is Tadpole Lake which most clearly illustrates the rôle of color in producing this wealth of sponge life.

Tadpole Lake, named for its shape, receives a small amount of seepage water through a long, gradually tapering slough which forms the "tail." The water analysis given in Table 1, which shows a color of 101, is the analysis of water from the "body". As one rows up the "tail", the water appears noticeably and progressively darker until, at a point where further progress by boat becomes impossible, the water appears fully as dark as the water of Helmet Lake. Concomitant with the increase in color is a marked increase in sponge fauna, especially *Spongilla igloviformis* and *Tubella pennsylvanica*. These species, ordinarily thought of as characteristic of obscure corners beneath their bases of support, now appear in the open covering every snag, and plainly visible at a depth of 2 to 10 cm. beneath the surface. When brought to the surface, *Spongilla igloviformis* invariably appears green, yet, seen through even a few centimeters of this dark water, it appears yellow to brown. No doubt it is to this color-absorbing quality of the water that these sponges owe their ability to live in exposed situations, and to the large amount of organic matter available as food, that they owe their luxuriant development.

The next darkest of the lakes (Harvey: color, 202; total organic 56.7 mgms. per 1) is given the following description in the author's field note book,

"A small lake with relatively large inlet and outlet, dark water, and bog margin. Several wagon loads of potatoes, recently dumped, float along the margin in various stages of decomposition. *Spongilla fragilis* exceedingly abundant coating sticks, dead wood, and even stones. Twigs of marginal Chamaedaphne completely coated where submerged, and, even above the water level, encrusted with dry sponge and gemmules." Although more massive specimens of *Spongilla fragilis* were found in other places, still no where else did the author find every available inch of support so completely occupied by this species as in Harvey Lake.

Little Pickerel, with color varying between 92 and 150, probably gives the nearest approach to perfection for the development of *Spongilla lacustris*, although the luxuriance of this species was practically equalled in another dark lake, Mary (color, 132).

The author does not, of course, wish to imply that color, or color and the high organic content which usually accompanies it, are alone responsible for the remarkable development of sponges in the lakes just described. The fact that of these dark lakes the sponge fauna of one was dominantly *Tubella pennsylvanica* and *Spongilla ingloviformis* of another *Spongilla fragilis*, and of the third *S. lacustris*, shows the operation of other environmental factors. Furthermore, Stella Lake, with a color of 101 and organic content of 35.5 mgms. per liter, but with a margin largely rocks and gravel, and with no protected sloughs or bays, had a relatively sparse sponge fauna despite its dark color. It is not, however, without significance, that the lakes supporting the greatest profusion of four species of Spongillidae were all dark in color.

DISSOLVED O_2 AND CO_2 IN WATER

Since dissolved O_2 and free CO_2, because of their relation to respiration, are universally conceded to be factors of primary importance for aquatic animals, they have been included here. Scrutiny of Tables 6 and 7 shows that the entire range of these factors encountered in the Wisconsin lakes under consideration falls within the limits of tolerance of all of the sponge

TABLE 6. The distribution of sponges with reference to the dissolved oxygen in the water

Sponge	Dissolved Oxygen as parts per million					
	5–5.9	6–6.9	7–7.9	8–8.9	9–9.9	10–up
E. mülleri	3	3	2	6	.	3
S. fragilis	3	5	7	19	1	5
S. lacustris	3	7	13	21	2	4
T. pennsylvanica	3	9	11	10	3	1
S. ingloviformis	.	5	3	2	.	.
E. everetti	1	2	5	7	4	.
S. lacustris (atypical)	1	2	7	5	3	.
Lakes	8	13	35	44	6	5

TABLE 7. Distribution of sponges with reference to free CO_2 in the water

Sponge	Free CO_2 as parts per million						
	0–0.9	1.0–1.5	1.6–2.5	2.6–3.5	3.6–4.5	4.6–5.5	5.6-up
E. mülleri	2	7	5	2	.	.	2
S. fragilis	10	11	12	3	2	.	3
S. lacustris	11	12	16	3	5	2	3
T. pennsylvanica	3	12	17	2	3	3	1
S. ingloviformis	.	3	4	1	2	2	.
E. everetti	1	11	9	1	.	.	.
S. lacustris (atypical)	1	12	6	.	.	1	.
Lakes	17	43	36	7	7	3	4

species studied. Neither O_2 nor CO_2 can then be regarded as influencing the distribution of sponges in the waters studied.

While, at a casual glance, Tables 6 and 7 may appear to indicate a restriction of *Spongilla ingloviformis* to certain concentrations of O_2, or of *Ephydatia everetti* to low CO_2, yet the facts of distribution, that neither of these forms was ever found associated with *Ephydatia mülleri* or *Spongilla fragilis* in nature, as well as the very small number of lakes in the part of the range from which they are absent, show that no significance can be attached to these apparent restrictions.

BOUND CO_2 IN THE WATER

Bound CO_2 is an index to the carbonate salts of the water, which consist largely of salts of Calcium. Birge and Juday (1930) show that the "hardest" water in the area studied is actually a "soft" water as compared to waters of the southern part of the state. It is, then, to be expected that the more widely distributed species, such as *Ephydatia mülleri* and *Spongilla fragilis*, would tolerate the upper limits of the bounds CO_2 found in this region.

Table 8 and Figure 1 indicate a definite restriction of *Ephydatia mülleri* to the upper part of the range of bound CO_2 found in the lakes studied, and of *Spongilla ingloviformis*, *Ephydatia everetti*, and the atypical *Spongilla*

TABLE 8. Distribution of sponges with reference to bound CO_2 of the water

Sponge	Bound CO_2 as mgms. per liter					
	0.5–1.0	1.1–2.5	2.6–5.0	5.1–10	11–20	21–up
E. mülleri	..	..	..	6	10	3
S. fragilis	..	1	2	9	21	10
S. lacustris	1	6	8	14	17	9
T. pennsylvanica	9	11	10	7	5	..
S. ingloviformis	4	6	4	..	..	..
E. everetti	9	8	4	..	..	..
S. lacustris (atypical)	7	9	2	..	..	..
Lakes	15	29	24	16	29	11

lacustris to very low concentrations. *Tubella pennsylvanica*, although more tolerant than the last mentioned species, is, nevertheless, restricted to the less hard waters (Fig. 1). This is in keeping with the findings of Old (1932) who describes this species as limited to waters of low temporary hardness. *Spongilla lacustris* alone seems to tolerate the entire range of bound CO_2 found in the lakes studied. While bound CO_2 may explain the separation in nature of *Ephydatia mülleri* from *Ephydatia everetti* and *Spongilla ingloviformis*, it does not explain the fact that *Spongilla fragilis* has not been found coexisting with either of these species, nor the complete separation of the microspined, from the non-microspined forms of *Spongilla lacustris*.

HYDROGEN ION CONCENTRATION OF THE WATER

TABLE 9. Distribution of sponges with respect to the pH of the water

Sponge	PH															
	5.0 5.1	5.2 5.3	5.4 5.5	5.6 5.7	5.8 5.9	6.0 6.1	6.2 6.3	6.4 6.5	6.6 6.7	6.8 6.9	7.0 7.1	7.2 7.3	7.4 7.5	7.6 7.7	7.8 8.1	8.2 up
E. mülleri	.	.	.	.	.	.	.	.	3	1	3	5	3	2	.	2
S. fragilis	.	.	.	.	.	1	.	2	3	3	4	9	6	5	3	6
S. lacustris	.	1	1	1	2	4	1	3	6	3	5	9	6	5	1	6
T. pennsylvanica	3	1	4	3	5	4	2	6	3	3	5	3	1	.	.	.
S. ingloviformis	2	2	3	2	2	2	1	.	.	.	.	.	.	.	.	.
E. everetti	4	1	2	2	6	4	2	.	1	.	.	.	.	.	.	.
S. lacustris (atypical)	2	.	3	3	4	4	1	1	1	.	1	.	.	.	.	.
Lakes	6	3	6	6	8	10	9	11	9	9	8	12	7	8	6	6

Of the species included in Table 9, *Spongilla ingloviformis* alone appears to be sharply restricted to the more acid waters, although *Ephydatia everetti* shows a marked preference for concentrations below pH 6.4 and *Tubella pennsylvanica* is lacking from the more basic waters. Old, in Michigan, found *Spongilla lacustris* and *Ephydatia mulleri* in pH 6.0 to 9.0, and *Spongilla fragilis* throughout the entire range 4.2 to 9.2. The fact that, in Wisconsin, *Spongilla lacustris* has been found extending farther into the acid range than *Spongilla fragilis*, may suggest that something other than pH is the controlling factor. pH may, however, be a factor in limiting the distribution of *Tubella pennsylvanica*, since, when collected from less acid waters, it was always found close to a mucky bottom—a position of local acidity not shown in the table.

SILICA CONTENT OF THE WATER

Because SiO_2 is the substance from which all freshwater sponges build their spicules, it may reasonably be an important factor in their distribution.

Ephydatia mülleri is apparently restricted to the higher concentration of silica. Characteristically this species has a strong skeletal development and

TABLE 10. Distribution of sponges with reference to the SiO_2 of the water

NOTE—In the determination of SiO_2, which is colorimetric, the lowest color standard used indicates 0.5 mgms. per liter SiO_2. Readings below this value are, therefore, not strictly quantitative, but depend upon the chemist's estimate of a very faint color.

Sponge	SiO_2 as mgms. per liter						
	0-trace	0.25–0.4	0.45–0.8	0.9-1.5	1.6-5.5	4.6–8	over 8
E. mülleri	.	.	1*	.	10	7	1
S. fragilis	.	1	3	3	18	13	4
S. lacustris	1	7	6	6	17	12	5
T. pennsylvanica	9	10	7	3	9	3	2
S. ingloviformis	4	5	2	2	1	.	.
E. everetti	13	7	2	.	.	.	.
S. lacustris (atypical)	13	6	.	.	.	.	.
Lakes	23	29	13	11	25	17	6

*Sponge weak and with sparse slender spicules—little St. Germaine Lake.

vigorous vegetative growth. It is the only branching form collected by the author which had sufficient rigidity of skeleton to hold its branches upright and maintain its shape when taken from the water (Fig. 4). The one specimen of this species collected from a water low in silica (Little St. Germaine, Sio_2 0.7) was so atypical as to be almost unrecognizable. This specimen was found on the under side of a floating log at the point where the lake narrows down to its outlet. Instead of the rich, strongly lobed to massive, vegetative growth usually associated with this species, it formed a delicate, transparent, whitish film through which the numerous, small, yellow gemmules could be plainly seen. The gemmule spicules, though sparse and scattered, were the short shafted birotulates characteristic of *Ephydatia mülleri*. The skeletal spicules too, although very slender, had the microspining characteristic of the species; so the identification was possible. The fact that the only specimen of this species collected from a water poor in silica was so extremely attenuated, is additional evidence that *Ephydatia mülleri* requires a moderate to high concentration of SiO_2.

Spongilla fragilis, although less restricted than *Ephydatia mülleri*, appears also to be limited by the silica content of the water. The few small specimens collected from lakes low in silica (George, Anna, Bug, and Little St. Germaine) showed weakly developed skeletal spicules, and gemmules almost or completely lacking the usual encrustation of acerates.

Spongilla lacustris, although present in both high and low silica waters, shows marked differences in skeletal development. When collected from a water high in SiO_2, as North Twin Lake, it has long, rigid, almost brittle, lobes which feel rough to the hand and are usually visibly bristling with spicules. In a lake low in SiO_2, as Lake George, the sponge frequently occurs in even greater abundance but the lobes are fleshy and soft. They feel slimy to the touch and collapse to form a shapeless mass when taken from the water. That the ability of the sponge to deposit silica is modified

by some constituent of the water other than silica itself, is suggested by the fact that the size of spicules in a large series of sponges examined was not strictly proportional to the silica content of the water, especially in waters very low in silica. When, however, a series was selected varying both in SiO_2 and other substances in solution as shown by the conductivity, the degree of skeletal development in the lower concentrations merged gradually into the extremely weak condition characteristic of the "atypical" or non-microspined form (Figures 10-16).

Except for differences in degree of skeletal development, *Tubella pennsylvanica* is apparently indifferent to the silica content of the water, since well developed colonies were found in waters of both high and low silica content.

Two species, *Spongilla ingloviformis* and *Ephydatia everetti*, are restricted to the waters of lower silica content. These species are uniformly weak in skeletal development, depending, for shape, upon a tough external membrane or the buoyancy of the water. Spicules are, apparently, not a necessity, but merely an incidental part of their structure since, unlike *Ephydatia mülleri* and *Spongilla fragilis*, specimens with weak spicule development frequently attain large size and numerical abundance. Even in these forms in which spicules might be regarded as unnecessary an increased silica content of the water is, however, accompanied by heavier spicules; thus *Ephydatia everetti* from Bear Lake (SiO_2, 0.8) exhibits much heavier spicules than the same species from Bass Lake (SiO_2, 0.2) or from lakes of still lower silica content. (Figures 2 and 3).

The fact that the "atypical" forms of *Spongilla lacustris* are sharply restricted to waters very low in silica is regarded by the author as a matter of cause and effect rather than of habitat selection. In other words, the low silica content of the water is probably one of the factors which caused those specimens to be atypical, or to fail to develop microspined dermals. That SiO_2 is not the only factor involved in this skeletal modification is indicated by the fact that the typical, or microspined, forms while never collected from the same waters were collected from waters as low in SiO_2 as some of the "atypical" forms.

Silica, then, appears as an important factor in determining the degree of skeletal development, both size and abundance of spicules, in all of the *Spongillidae* studied. Its scarcity, moreover, may be a limiting factor in the distribution of strongly skeletoned forms such as *Ephydatia mülleri* and *Spongilla fragilis*.

RESIDUE AND CONDUCTIVITY OF THE WATER

The total amount of solids in solution in the water, and so left as a residue when the water is evaporated, constitutes the "residue". The residue thus contains both inorganic and organic matter in varying amounts. "Conduc-

tivity", or the readiness with which the water conducts an electric current, depends upon the ionized material present in the water, mostly inorganic salts, and so constitutes an index to the total amount of soluble inorganic matter present. Juday and Birge (1933) have shown that, for the Wisconsin lakes, conductivity is dependent largely upon the salts of calcium and magnesium present, and so correlates closely with bound CO_2, although not identical with it.

TABLE 11. Distribution of sponges with reference to the residue of the water

Sponge	Residue as mgms. per liter							
	11–15	16–20	21–30	31–40	41–50	51–60	61–70	Over 70
E. mülleri	.	.	.	.	5	5	4	5
S. fragilis	.	.	1	2	6	11	10	13
S. lacustris	.	.	6	5	9	12	8	12
T. pennsylvanica	9	7	5	2	7	5	2	5
S. ingloviformis	4	2	.	1	3	1	.	1
E. everetti	12	5	5	.	.	.	.	.
S. lacustris (atypical)	11	7	1	.	.	.	.	.
Lakes	17	20	15	11	12	17	12	16

TABLE 12. Distribution of sponges with reference to conductivity of the water

Sponge	Conductivity as reciprocal megohms							
	7–10	11–15	16–20	21–30	31–50	51–75	76-100	over 100
E. mülleri	.	.	.	.	4	10	5	.
S. fragilis	.	.	3	1	6	20	10	2
S. lacustris	.	1	14	4	9	18	9	2
T. pennsylvanica	6	12	11	3	4	6	.	.
S. ingloviformis	4	4	6	.	.	.	.	.
E. everetti	10	12	1	.	.	.	.	.
S. lacustris (atypical)	6	12	1	.	.	.	.	.
Lakes	13	30	21	7	11	28	11	2

A sharp division is shown among the sponges with reference to both of these factors, residue and conductivity, a division, moreover, which shows close correspondence to the way they are associated in nature. For both factors, *Ephydatia mülleri* and *Spongilla fragilis* are restricted to the moderate to higher concentrations, whereas *Ephydatia everetti* is even more sharply restricted to the lower concentrations. Juday and Birge (1933) find that ordinary distilled water has a conductivity of from 3 or 4 to 9, whereas special precautions such as distillation in hard glass are necessary to produce a water with conductivity below 3. Lake waters, then, with conductivity of 7 to 12 are almost as free from minerals as much of the ordinary distilled water. From this we can obtain an idea of the probable sensitivity of a sponge such as *Ephydatia everetti*, which attains its best development in waters having less than 20 mgms. per liter residue and a conductivity below 15. For both

factors, the typical form of *Spongilla lacustris* is tolerant of all but the lowest concentrations whereas the atypical form is restricted to very low concentrations, a highly suggestive fact, although neither residue nor conductivity show a sharp enough distinction between the concentrations tolerated by the two forms to fully explain the fact that they were never found co-existing.

Tubella pennsylvanica appears to be tolerant of the entire range of residue. The absence of this species from 4 lakes having residues above 79 can not be regarded as significant. It is probably limited by the higher conductivities, a fact in harmony with its observed abundance in waters high in organic content and its sensitivity to calcium.

Spongilla igloviformis alone appears indifferent to residue but sharply limited by conductivity, since it occurs throughout almost the entire range of the former but is limited to very low conductivities. This restriction to waters of low conductivity would account for the fact that this species was never found associated with *Ephydatia mülleri* or *Spongilla fragilis*, whereas the tolerance for waters of high residue would explain the only moderately frequent association with *Ephydatia everetti*.

SUMMARY OF CHEMICAL DATA

To return to the questions raised at the beginning of the discussion, it appears: (1) that the factors favoring *Ephydatia mülleri*, *Spongilla fragilis*, and *S. lacustris*, but inimical to *Ephydatia everetti*, are both high mineral content (Bound CO_2, SiO_2, Conductivity, and Residue in part), and high organic content (accompanying high color and high residue); (2) that the factor inimical to *Ephydatia mülleri* and *Spongilla fragilis*, tolerated to a slight degree by *Spongilla lacustris*, but favorable to *Ephydatia everetti* is the extremely low mineralization of the water or absence of solutes, and (3) that the factors favoring *Spongilla igloviformis* and *Tubella pennsylvanica*, but inimical or indifferent to the remaining forms so far as their presence or absence is concerned, are high color and high organic content. Also that sensitivity to mineral content (indicated by bound CO_2 and conductivity) and to low hydrogen ion concentration (indicated by high pH) may serve to restrict the distribution of these two last named species. The problem of the complete non-association of the microspined and non-microspined forms of *Spongilla lacustris* remains incompletely solved.

Of the physical and chemical factors thus far discussed, four, (Bound CO_2, SiO_2, conductivity, and residue) have shown some degree of correlation with the dissociation of the "typical" and "atypical" forms of *Spongilla lacustris*, however no single factor has shown such perfect correlation as to indicate it as the sole cause of the phenomenon. It appears possible that the explanation may be found in a combination of these four factors. It is well known that among higher animals the ability of an organism to utilize certain food essentials, as calcium, even when provided in abundance, depends upon

the presence also of certain other foods. So it may be that with sponges the ability to form spicules depends not only upon the supply of silicon, but also upon certain other foods associated with the other solutes of the water.

In Table 13 an attempt has been made to secure a single numerical index to all four factors by taking their sum. Because the importance of SiO_2 is obviously disproportionate to the minute quantities found in water, the SiO_2 values have been multiplied by 10. The other values are as given in Table 1.

TABLE 13. Distribution of sponges with reference to the sum of bound CO_2 and residue as mgms. per liter; conductivity as reciprocal megohms, and SiO_2 as mgms. per liter times 10

Sponge	Bound CO_2, Residue, Conductivity and SiO_2 x 10									
	21–25	26–30	31–35	36–40	41–50	51–75	76-100	101–150	151–200	200-up
E. mülleri	.	.	.	.	.	.	.	3	8	6
S. fragilis	.	.	.	.	.	3	.	8	15	17
S. lacustris	.	.	.	.	4	8	5	8	13	15
T. pennsylvanica	2	6	4	4	3	6	3	7	4	3
S. ingloviformis	.	3	3	1	.	2	3	1	.	.
E. everetti	3	8	5	2	2	.	.	.	.	.
S. lacustris (atypical)	3	6	6	4	.	.	.	.	.	.
Lakes	4	11	9	7	5	9	5	10	17	20

Table 13 shows a sharp separation between the typical and atypical forms of *Spongilla lacustris* found in nature. Furthermore, although *Tubella pennsylvanica* is present throughout practically the entire range, 15 of the 16 lakes from which forms with extremely attenuated spicule development (the "minima" variety) were recorded, are represented in the first four columns of this table; whereas the sixteenth lake, and three of the four lakes listed as containing transitional forms, are included in the fourth and fifth columns (between 37 and 44). It therefore appears that the sum total of solutes in addition to the especially important silicon compounds determine the degree of development of spicules in *Spongilla lacustris* and *Tubella pennsylvanica,* although not their presence or absence.

OPTIMUM HABITATS

Three methods have been frequently used for determining the optimum habitat of animals from purely field data: (1) the absolute frequency of occurrence of the species in question in the various habitats studied; (2) the relative frequency of occurrence, or the frequency of occurrence of the organism over the frequency of occurrence of the environment; and (3) the selection, as an optimum environment, of the conditions under which the species was found most perfectly developed and in greatest abundance.

That the first of these methods may be entirely misleading, is evident if we consider, for example, the distribution of *Tubella pennsylvanica* with

respect to the color of water. This species was collected 18 times in waters with colors below 20, and only 10 times in waters with colors above 100, a fact which would suggest its optimum habitat as uncolored waters. When, however, we note that 59 of the lakes examined had color values below 20, whereas only 13 had color values above 100 (that 30% of the lakes with low color supported this sponge as compared to 77% of those of high color) the fallacy of the method becomes apparent.

The habitat selection of the sponges as judged by their relative frequency may be quickly determined from the tables by comparing the frequency of occurrence of the sponge in any given type of water, with the frequency of occurrence of lakes containing that type of water. In Table 14 the author has used the third method of describing an optimum habitat, the range of conditions in the lakes in which each species was found in greatest luxuriance and profusion. The lakes selected as optimum habitats are as follows (See Table 1):

For *Ephydatia mülleri:* Little Rice, Little Papoose, Tamarack.

For *Spongilla fragilis*: Harvey, Turtle, Little Arborvitae, Little Rice, Dam, Rice.

For *Spongilla lacustris:* Little Pickerel, Rose, Mary, George, Anna.

For *Tubella pennsylvanica:* Helmet, Tadpole, Nellie, Helen, Harvey.

For *Spongilla ingloviformis:* Helmet, Tadpole, Yolanda, Malby, Elizabeth.

For *Ephydatia everetti:* Bass, Larry, Joyce, Louise.

For *Spongilla lacustris* (atypical): Joyce, Larry, Carlin, Katinka, Clear, Walker.

TABLE 14. Range of optimum conditions for Wisconsin *Spongillidae*

Sponge	Environmental conditions						
	Color	Free CO_2	Bound CO_2	pH	SiO_2	Residue	Conductivity
E. mülleri	43–101	1–3	8. 4–20	7.0–7.2	2. 1–7.0	45–75	45–78
S. fragilis	26–202	0.5–13.9	6. 0–21.5	6.6–7.4	2. 0–8.2	45–78	33–78
S. lacustris	43–150	1.0–5.5	2. 0–8.0	5.4–6.8	0. 3–12	33–70	19–28
T. pennsylvanica	97–268	0.5–5.0	0.68–7.0	5.6–6.6	0. 4–4.4	39–78	10.8–35
S. ingloviformis	6–268	1.0–5.0	0.88–3.0	5.0–6.0	0.25–4.4	14–71	11–20
E. everetti	6–12	1.2–3.0	0. 7–4.5	5.1–6.0	Tr. –0.3	15–18	9–13.5
S. lacustris (atypical)	0–35	0.5–2.0	0. 7–4.5	5.1–6.4	Tr. –0.2	15–22	8–17

EFFECTS OF CURRENT AND WATER MOVEMENT

In addition to chemical content, water movement is also of importance in sponge distribution. To *Spongilla fragilis*, and probably also to *Ephydatia mülleri*, it appears to be of considerable importance. The rankest growths of both of these species observed by the author were found in the Wisconsin River and its tributaries, Mud Creek and Rice Creek, in the expanded and

relatively quiet stretch above a power dam west of the village of Eagle River. The construction of the dam had inundated a considerable area of shrubs and trees, many of which had since fallen over and thus provided a large surface for attachment of sponges, as well as an increased organic content due to their decomposition. The water, although without perceptible current, had sufficient movement and interchange with the main channel to prevent stagnation. In this region literally wagon loads of sponges could have been collected. The advantage of gradual water movement over rapid current was evident from a comparison of this stretch of the river with a rapids in the same river a few miles below. The water was essentially the same at both places, no tributaries having entered between. In the rapids the same two species of sponge were still present, encrusting the protected sides of rocks, but the colonies, although numerous, were inconspicuous and small,—rarely over 2 cm. in diameter.

Of the six lakes selected as supporting *Spongilla fragilis* in greatest abundance (Table 14), four had inlets and outlets large enough in proportion to the size of the lake to insure a gradual but constant movement of water through the lake, while in the other two, the profusion of sponges was found where the lake narrowed down to a strait (Turtle Lake) or outlet (Little Arborvitae) so that locally there was continuous water movement. Similar conditions maintain in two of the three lakes where there were optimum growths of *Ephydatia mülleri.*

Spongilla lacustris, although tolerant of current, as evidenced by the fact that it was collected from 14 of the 17 streams examined and was taken from rocks in rapids, nevertheless was found most luxuriantly developed and most abundant in a group of lakes high in organic content but without inlet or outlet, at least during a large part of the season. Old (1932a) collecting from 84 streams and 61 lakes and ponds, found both *Spongilla fragilis* and *Ephydatia mülleri* more common than *Spongilla lacustris*, whereas the author, collecting primarily from lakes found *Spongilla lacustris* by far the most common, a fact which again illustrates the effect of water movement in the distribution of these forms.

Tubella pennsylvanica and *Spongilla ingloviformis* are distinctly quiet water species. Of the 46 lakes supporting *Tubella pennsylvanica* and 15 supporting *Spongilla ingloviformis*, 36 and 15 respectively are purely seepage lakes. The former sponge, although taken also from streams, was invariably found in quiet protected places where boggy conditions prevailed.

Ephydatia everetti was confined to seepage lakes. This fact can not, however, be interpreted as showing intolerance for current, since only lakes of the seepage type have water of the extremely low chemical content apparently demanded by this species.

Of the sponges less frequently found, *Heteromyenia argyrosperma* was

collected five times each from lakes and streams. Of the collections from lakes, four were made either near the outlet or at the mouth of an inlet where continuous exchange of water would be assured. *Heteromyenia repens* was collected five times from lakes and once from a stream, the water movement varying from the stagnation of a bog (Bug Lake) to a stony riffel. *Heteromyenia ryderi* was taken once each from a slow moving stream and a bog lake, although very poorly developed in the latter. *Carterius tubisperma* was collected twice from riffels and once from a lake at the head of its outlet where continuous water movement was assured. Old also lists this species as preferring streams.

From the observations described it would appear that *Spongilla fragilis*, *Ephydatia mülleri,* and *Heteromyenia argyrosperma,* although tolerant of quiet water, are favored by continuous water movement, that *Spongilla lacustris,* although tolerant of current, is primarily a quiet water species, that *Tubella pennsylvania* and *Spongilla igloviformis* show a distinct preference for quiet water, whereas *Carterius tubisperma* as distinctly prefers running water.

ECOLOGICAL VARIATIONS OF TAXONOMIC CHARACTERS

Growth Form

Growth forms of sponge colonies have been frequently discussed from the point of view of their use in field recognition of species, or as criteria of sub-species or varieties, though, at the present time, most workers recognize both colony form and texture as too variable to be of taxonomic value.

Old (1932a) summarizes his observations as follows: "Branched colonies are not always *S. lacustris* and colonies of *S. lacustris* are not always branched. Branched colonies are found in *S. aspinosa* and *H. repens.* Short, blunt, finger-like projections are found in *E. mülleri, H. ryderi, H. argyrosperma* and *C. tubisperma.* The writer found unbranched and branched colonies of *S. lacustris* side by side in lakes and streams. The reason for this is not known. . . . Unbranched colonies were held by Annandale (1911) to be the result of unfavorable conditions. This can hardly be true when both forms are found side by side in the same water on similar supports."

From the author's observations it seems that, although certain colony forms may be characteristic of certain species, that no species is limited to any particular form of colony, and that wide variations from the so-called "characteristic forms" are produced by both physical and chemical factors of the environment as well as by age.

Of the species repeatedly collected by the author, one only, *Tubella pennsylvanica,* adhered strictly to a purely encrusting type. Pseudo-branching was common where this sponge had enveloped water roots or twigs; the depth of the colony varied from a delicate, semi transparent film to an incrustation as

much as 10 mm. thick, but no lobed or branched specimens were, at any time, observed.

Spongilla ingloviformis, although usually encrusting, becomes rugose to irregularly lobed (Fig. 8 & 9) under apparently its optimum condition of growth.

Spongilla fragilis, from Wild Cat and Mann lakes, two of the lakes of highest mineral content, formed a firm, almost uniform, incrustation of fine texture and with small, regular, slightly protruding, oscula. In Trout River, Turtle Lake, and Little Arborvitae, the growth was much more robust, the texture looser, the surface less regular, and the oscula larger and more protruding (Fig. 7), whereas, under conditions of its most luxuriant growth found above the Wisconsin River dam, the surface developed irregular lobes and protrusions suggestive of incipient branching (Fig. 5 & 6). Although fineness of skeletal texture and regularity of pores have both been used as criteria of varieties or sub species in *S. fragilis*, the author is inclined to regard them as influenced both by current and by the relative abundance of skeleton-building materials and organic foods, an irregular surface resulting from a rapid growth produced by high organic content, in the absence of a proportionately high mineral content for skeleton building.

Spongilla lacustris exhibits every variation in form from simple encrustations to tufts of long, slender, finger-like processes. Although no single factor is responsible for the entire range of forms, the age of the colony, area and slope of the surface to which it is attached, wave action, and chemical content of the water, are all regarded as influencing the growth-form of this species. Repeated observation of the same colony during the summer of 1932 showed that colonies which were encrusting in early July had become abundantly branched by the third week of August, whereas younger colonies on the same log, not observed at all at the time of the first visit, were still encrusting; a fact which may explain the observation of Old (1932a), that branching and encrusting colonies may be found side by side in the same habitat. Encrusting sponges are more frequently found on broad surfaces, such as planks or large logs, than on slender attachments, such as twigs. They are also commoner on the under side of the support than on the upper side. This latter condition was well illustrated by observation of the sponges in a pile of old timber in Little Rice Lake. Numerous large colonies of *S. lacustris* were found encrusting the under sides of these timbers, but only beginning to branch along the edges where the colony was growing in a vertical direction. Colonies on the upper sides of similar timbers began their branching in the central, or older, portion rather than at the edges. That this inhibition to branching was the result of gravity or the inverted position, rather than of shade, is evidenced by the fact that colorless but branching colonies have been collected from under bridges and other shaded locations.

Water movement, unless strong, has no apparent effect upon the branching of *Spongilla lacustris*. Except right in riffels, where only encrusting forms are found, the sponges of streams show the same type of branching as those of the lakes drained by the streams. Strong wave action may inhibit the development of branches. An excellent illustration of the varieties of growth form produced by physical factors was observed in Lake Anna. Here a large tree trunk almost covered by *S. lacustris,* sloped gradually from above the surface to a depth of about 2.5 meters. The situation, in the lee of an island, although not completely protected from wind, was sheltered from strong wave action. The sponges were encrusting on the under side of this log; they became irregular and lobed as they rounded the sides, and strongly branched on the upper surface. Another gradient was observed along the upper side of the log, from the water's edge to below wave action. Near the surface of the water the branches were short, thick, and irregular knob shaped; at two decimeters depth they were 4 to 5 centimeters long and somewhat club shaped, whereas at a depth of 0.5 to 1.5 meters they were of the typical finger shape, and 10 to 12 centimeters long. Vaughan (1919) describes a similar relationship between growth forms of corals and depths of water and violence of wave action. Specimens from waters high in organic matter tend to have numerous long, fleshy, closely crowded and frequently anastomosing branches, whereas specimens from waters low in organic matter but high in mineral content are more sparsely branched and the branches are independent, more slender, and of more uniform diameter. The extreme of this latter type was collected from North Twin Lake, a small, shallow, bog lake with a barren peat bottom, but very high in silica. In this lake the *Spongilla lacustris* bore branches 3 to 4 decimeters long but only 7 or 8 mm. in diameter, and not more than 2 to 3 branches to one small basal part. The long branches, in part reclining on the bottom and in part ascending, might better be described as worm-shaped than as finger-shaped. Their texture was poor in fiber but so rich in spicules as to render them brittle and "scratchy" to the touch.

Ephydatia everetti (Mills) 1884 is described by Potts as "without sessile portion, but consisting altogether of slender meandering filaments little more than a sixteenth of an inch in diameter." Although this describes the growth form of *E. everetti,* when collected from its usual attachment (aquatic vegetation such as Eriocaulon or Isoetes) or from an irregular surface, still specimens having an extensive sessile portion, and even encrusting colonies, have occasionally been taken in the same locations, from larger more uniform attachments such as fallen trees.

While, in general, it appears that after a sponge has once encrusted its base of support, a rapid growth tends to produce an irregular, undulating, rugose, lobed, or branching surface, still there are so many factors affecting

growth form that any statement to the effect that encrusting colonies indicate unfavorable conditions, are an adaptation to current, are an abnormality, or even are younger, seems wholly unwarranted.

SPICULE DEVELOPMENT

FIELD OBSERVATIONS

Since the classification of the *Spongillidae* is based largely upon skeletal parts, especially the spicules surrounding the gemmules, any variation in spicules produced by the environment is certain to be of taxonomic, as well as of ecological interest.

Under the discussion of SiO_2 it was pointed out that, in waters of low SiO_2 content, the size and number of spicules is greatly reduced; that in *Ephydatia mülleri* and *Spongilla fragilis* the spicules associated with the gemmules tend to disappear, and the entire skeletal structure to become delicate and attenuated, in waters of mineral content near the lower limit of tolerance of the species; and that similar variations have been observed in other species.

Figures 2 and 3 show gemmule, dermal, and skeletal spicules of *Ephydatia everetti* from waters having 0.2 and 0.8 mgms. per liter SiO_2 respectively. The vegetative parts of these two sponges were equally thrifty and well developed. If spicule measurements can be accepted as a criterion of varieties, one can find here a variety difference, however a complete series connecting these two can be established by considering materials from the entire 22 lakes from which this species was collected.

Figures 10 to 16 are microphotographs of the spicules of *Spongilla lacustris* collected from seven different lakes (North Twin, Papoose, Rock, Nebish, Mary (near Papoose), Katinka, and Joyce) arranged in order of descending silica content. The magnication is the same throughout the series. From North Twin to Nebish a progressive attenuation of spicules, both skeletal and dermal, is apparent. The skeletal spicules in the latter, although longer, are no greater in diameter than the dermal spicules in the former. The dermal spicules, while still present, and still microspined, in the specimen from Nebish, require careful adjustment of the microscope and light to ascertain the fact.

From this observation as to the correlation of spicule reduction and decrease in skeleton forming material in the water, one would expect that a still further decrease in SiO_2 might reduce the skeletal spicules and, eventually, eliminate the dermals altogether. In this event, one should expect the skeleton-poor *Spongilla lacustris* to look just like the "atypical" specimens of *S. lacustris* shown in the next two figures (Figures 14 and 15). The spicules from Mary Lake (Fig. 14) show a condition frequently found in these sponges from soft-water lakes, an abundance of spicules variable in

size and intermediate in character between skeletal and dermal spicules. Similar spicules were described by Potts, 1887, for *Spongilla aspinosa.* In Fig. 15, the spicules from Lake Katinka, the group of small transparent spicules at the upper left of the picture are apparently of organic material, either lacking silica or so poor in that substance that they do not refract light as do ordinary spicules. The spicules from Joyce Lake (Fig. 16) show about the last stage in spicule reduction, as here, not only the dermals, but frequently even the skeletal spicules, are poorly or incompletely silicified and reduced to the verge of total disappearance.

While these seven types apparently form an intergrading series, and while the series would be still more complete and minutely intergraded if all of the specimens collected were considered, still, in as much as the presence of microspined dermal spicules is a species characteristic of *Spongilla lacustris,* the absence of these dermals places the sponges shown in the last three figures into a separate species, either *Spongilla aspinosa* Potts or a new species. At least, had the author's collections been less extensive, she would not have questioned the presence of two distinct species. It is possible, of course, that the difference between the sponges possessing and those lacking microspined dermals may be genetic, thus indicating two different species; yet, the field data all indicate a single species variously modified by environment. This latter interpretation is indicated by (1) the intergradation of the two forms as has been described, (2) the fact that the two forms, although both abundant in the limited area studied, have never been found coexisting in the same body of water, (3) the apparent correlation between degree of spicule development and the mineral content, especially SiO_2, of the water, and (4) the fact that a fluctuation in the water of one lake was accompanied by a change in the type of sponge found in it. Trilby Lake (Table 1), in 1932, showed no detectible amount of SiO_2. At this time the extreme soft-water or "atypical" form of sponge was collected in abundance. Two years later, a very dry year, the SiO_2 had increased to 0.5 mgms. per liter accompanied by a doubling in the amount of carbonates. At this time, not a specimen of the sponge lacking microspined dermals could be found; the sponges, although as abundant as formerly, were all the "typical" *Spongilla lacustris,* similar in spicule development to those from Nebish Lake (Fig. 13).

The extreme variations in *Tubella pennsylvanica* have already been mentioned. Between the extremely attenuated forms described earlier in this paper and the "typical" forms, were a great variety of modifications (Figures 17-22) including irregular, incised, and star shaped, rotules (both distal and proximal), and proximal rotules with rays or "spokes" of greater thickness from the shaft to the margin, and with lobed margins. Potts (1887) describes a somewhat similar series of variations found by him in *T. penn-*

sylvanica. Beginning with Bear Lake, an upper source of the Lehigh River at an altitude of 1800 ft., he found an extremely delicate form designated by him as *Tubella pennsylvanica var. minima.* Collections made from the same river at altitudes of 1200, 1000, 600, and 40 to 50 ft. showed progressively heavier spicules and closer approximation in size of the two rotules. According to Potts, "These changes followed closely the lines of increasing altitude; their *cause* must be left for later determination."

If, now, the Tubellas collected in Wisconsin, are arranged not in order of altitude, which varies but little in this region, but in order of increasing mineralization of the water, one can construct a series similar to that of Potts. Beginning with such lakes as Little Rudolph or Little John, Jr., in which the characteristics of Potts' "minima" are equalled or surpassed, and ending with the outlet of Mann Lake, where the heavily spiculed sponges may be regarded as "typical," we have all except the extremely heavily spiculed forms found, by Potts, near the coast. The explanation is doubtless the same in the two series, an increase in SiO_2 and mineral content of the water. Mountain lakes, which form the very head-waters of rivers (such as Bear Lake studied by Potts) are usually bodies of pure, soft water. As the stream winds its way to the coast, augmented by drainage and ground waters, it becomes progressively more highly mineralized, and such variations in the mineral content of the water would be reflected in the skeletal development of the sponges growing in it.

EXPERIMENTAL STUDY

Although field data indicate, in case of both *Spongilla lacustris* and *Tubella pennsylvanica,* that the wide range of spicule forms observed was the product of variety in environments, still, before any definite conclusion could be drawn it remained to test experimentally whether environment alone could produce such extreme variations as were found in nature. To make this test colonies of both species were transferred from Little Rudolph Lake; (conductivity 5.6 to 8.2 and SiO_2 merely a trace) a water from Trout Lake, (conductivity 75 and SiO_2 7.3) and the spicule modifications observed by examination of fragments of the colonies from time to time. For the purpose of the transfer a clean wooden barrel of 15 gallons capacity was used. In preliminary experiments, in which galvanized iron tubs were used, the sponges invariably died. The barrel was soaked a week in Trout Lake, then sunk three fourths of its depth in the sand to guard against rapid changes in temperature, and filled to about a third of its depth with sand. Fresh water from Trout Lake was pumped into the barrel mornings and evenings, and, during very hot weather, at three hour intervals during the day. As the only sponges found in Trout Lake had been collected from a bay at the head of its outlet almost two miles distant from the Biological Survey

headquarters, the chance of introducing sponge fragments or gemmules with the water was regarded as negligible. In order to provide a water of higher organic content than that of Trout Lake, about two gallons of bog water was added each time the water in the barrel was renewed. The Forestry Bog, from which this water was secured, had a conductivity of 9.5 to 12.0, SiO_2 1.4, and color of 70, and was without sponge fauna. A typical analysis of water from the barrel showed free CO_2 4.75, bound CO_2 9.5, pH 6.9, SiO_2 3.0, conductivity 40, and color 38.

In transferring the sponge colonies from Little Rudolph Lake care was taken that they should never be lifted from the water. As controls a piece of each colony transferred was removed for examination at the beginning of the experiment, and marked colonies left in Little Rudolph Lake were collected for examination at the close of the experiment.

DATA

The experiment was begun July 18 and terminated August 20, 1934. Fragments of the transferred colonies were examined July 28 and August 11 as well as at the beginning and close of the experiment. No change in spicule form occurred in the colonies left in Little Rudolph Lake as controls during the 33 days of the experiment. In each of the colonies transferred, however, the type of spicules changed completely, from the attenuated forms characteristic of extremely soft waters to the more robust forms usually found in waters of moderate mineralization. (Figures 20-25).

Detailed examination of several of the colonies made at the close of the experiment showed that the older parts of the colony, the parts matured before transfer from Little Rudolph Lake, still had the extremely slender type of spicules; the new parts of each colony, the advancing edges and newly formed lobes, had only the robust spicules formed in the water of higher silica content, whereas the zone of transition between old and new structure, the areas which were at the very edge of the colony at the time the transfer was made, showed a mixture of the two types of spicules. This would indicate that spicules, when once formed, can not make a second growth ever under conditions of abundant building material.

The extent to which the character of the spicules was altered by a change in the mineral content of the water is best seen by a study of Figures 18 to 25. The magnification is the same for all figures on this page. Figure 18 is a group of gemmule birotulates and the tip of one skeletal spicule of *Tubella pennsylvanica* from Little Rice Lake, a lake comparable to Trout Lake in silica content of water, but higher in organic content, hence more favorable for this species. The figure shows both the shape and overlapping arrangement of gemmule birotulates usually found in this species. Figure 19, a gemmule birotulate from a specimen collected from Percy Lake, shows the reduced distal rotule, long slender shaft, and lobed proximal rotule fre-

quently observed in material collected from soft water lakes. Figures 20 and 21 show gemmule birotulates from control material collected from Little Rudolph Lake. These are characterized by incised to stellate proximal rotules, knob shaped to stellate distal rotules, long slender shaft, and sparse distribution in the gemmule wall (Fig. 21). Figure 22 is a group of skeletal spicules from the same colony. In contrast to Figures 20-22, Figure 23 shows a group of spicules from the same colony after 33 days under the conditions of the experiment. The material on this slide was taken from the region of transition, or the part of the colony which was still developing at the time the sponge was transferred to a more highly mineralized water. The gemmule birotulates shown are all from the new growth and are comparable to those shown in Figure 18. The contrast between skeletal spicules developed in Little Rudolph Lake and those developed after transference to a water of higher silica content is brought out by comparing the acerate spicules in the center of the figure with those at the extreme right and left.

Similar modifications of the spicules of *Spongilla lacustris* are illustrated in Figures 24 and 25. Figure 24 shows three of the skeletal spicules and a group of the minute nonspined dermal spicules from a control specimen from Little Rudolph Lake. Figure 25, a microphotograph from the transitional part of the same colony after development in the experimental water, shows one microspined dermal spicule (in the upper part of the figure) and part of one skeletal spicule (diagonaling the figure from upper left to lower right) belonging to the new growth, as compared to a group of spicules (lower part of the picture) developed before the transfer was made. It will be observed that the dermal spicule developed under the conditions of the experiment is of greater diameter than the skeletal spicules developed in Little Rudolph Lake. Although the magnification is greater, a comparison of the spicules formed after the transfer with the spicules of sponges collected in nature from water of moderate hardness (Figs. 10-12) shows that they are in every way comparable.

It can be concluded, then, on the basis of both field observation and experiment, that the great variety of spicule sizes and forms exhibited by both *Tubella pennsylvanica* and *Spongilla lacustris*, represent somatic modifications produced by the environment, not incipient species. Furthermore, in as much as this great variety of spicule-form is apparently produced by a variety of natural conditions under all of which the sponge is able to grow and reproduce normally, it follows that no one type of spicule can be selected as "typical" or "normal" and the others regarded as "abnormal". Moderately hard waters are more common than waters of conductivity below 20; the types of structures they produce are, therefore, more common; this does not, however, make them any more normal. They are all normal responses of a normal organism in one of its normal natural environments; and any adequate taxo-

nomic definition made for such a variable species must be sufficiently flexible to include its normal natural variations.

A Question of Nomenclature

Although the purpose of this study has been purely ecological, the necessity of using names with which to designate the sponges collected has forced the author to make one decision for herself in the field which belongs primarily to taxonomy. As in using the name "*Tubella pennsylvanica* Potts, 1882", the author is at variance with at least two contemporary workers on the *Spongillidae*, who designate the same species as "*Trochospongilla pennsylvanica* (Potts) 1882", some explanation of this adherence to the older name seems in order.

The Genus Tubella, established by Carter in 1881, was based upon four South American species, all characterized by gemmules having a granular crust charged with trumpet-shaped, inaequibirotulate spicules, the larger rotule of which rests upon the chitinous coat; the size of the outer rotule smaller, but having a variable relation to that of the former. In all four of the species placed in this genus by Carter, the larger rotule is described as having an even circular margin; although in two, the smaller rotule is described as toothed.

The following year, a fifth species, *Tubella pennsylvanica,* was described from North America by Potts. During the five succeeding years the same author received specimens of this species from many parts of this continent, including Newfoundland, and, by comparison of a large series of specimens, was able to describe many variations found within the species. These variations included not only extreme differences in robustness of spicules and relative diameter of the proximal and distal rotules, but even a toothed or rayed condition of the smaller rotule, and, in one form, a division of the larger rotule into uneven rays or rounded segments. Thus the extreme variability of *Tubella pennsylvanica* was clearly stated by Potts as early as 1887.

The Genus Trochospongilla was established by Vejdovsky in 1883, for two species having "amphidiscs smooth with entire margins", and, again, he described the gemmules as "covered with spool-like amphidiscs whose rotules have entire margins".

Potts (1887 and also 1918) accepts Vejdovsky's genus, and makes it clear that Trochospongilla is distinguished by entire margined *equal* rotules, whereas Tubella has more or less *unequal* rotules, which are usually, but not uniformly, entire, and that the shaft connecting the rotules is, in Tubella, more or less swollen at the end adjoining the proximal (larger) rotule.

Annandale (1911) redefines the genera Tubella and Trochospongilla, and places Potts' species in the latter genus, making it *Trochospongilla pennsylvanica*. According to his definition, the genus Trochospongilla is charac-

terized by its smooth margined birotulates, irrespective of variations in diameter, whereas "Tubella" is reserved to designate forms in which the unequal birotulate discs differ not only in size but also in form; the smaller disc is a rounded knob, the larger serrated and flat. In making this redefinition, Annandale completely ignores the numerous variations in form of rotule existing in the species under question.

Gee (1932 and 1933) accepts the revision of Annandale with the added qualification that the trumpet-like birotules of Tubella have comparatively longer shafts than those of Trochospongilla. In discussing his materials of *T. pennsylvanica*, Gee says, "While the rotules are generally circular, yet asymmetrical ones frequently occur and I have found some of the small rotules to be distinctly incised; also the lower rotules in some specimens recently examined have decidedly irregular edges which frequently are provided with rounded incisions or lobes rather than angular indentations. This state of affairs raises the question as to whether this form may not really be a connecting link between the *Tubellas* and the *Trochospongillas*." Again, after figuring distinctly toothed distal rotules, he says, "This last mentioned characteristic brings this form very close to the genus *Tubella* and leads us to wonder if it may not be a more or less transitional form connecting up the two genera *Trochospongilla* and *Tubella*. It is possible to find connecting links between the extremes and we know therefore that they represent the same sponge."

After examining 192 different specimens from 46 lakes and 3 streams of northeastern Wisconsin, the author finds, in that limited area, practically all of the various modifications and variations described by Potts and by Gee, as well as specimens in which the birotulates vary greatly in relative length of shaft as compared to diameter of rotules, and specimens in which the larger rotule is distinctly incised. Having found all intergradations, the author, like Gee, is convinced that a single species is represented. In addition, the author finds a distinct correlation between certain of these variations in structure of spicules and the chemical content of the water from which the sponge was collected. Instead, then, of being interpreted as incipient species connecting the genera Trochospongilla and Tubella (as Gee would apparently interpret them) these variations are regarded as ecological varieties; somatic modifications produced by the environment. If this interpretation is accepted and Annandale's separation of the genera Tubella and Trochospongilla on the basis of the margins of the rotules is recognized, one is confronted by the absurdity of supposing that the kind of water a sponge grows in can change its genus without changing its species. To avoid this dilemma, the author reverts to the generic definitions accepted by Potts. The genus Tubella is then distinguished by inequality of rotules and greater diameter of the shaft near the proximal rotule, whereas the genus Trochospongilla has equal rotules

with the ends of the shaft similar. If these generic criteria also prove "shifting sand", then by the law of priority, it is the genus Trochospongilla which must pass into synonymy; but, in either event, the species under question remains as originally named, *Tubella pennsylvanica* Potts 1882.

There is one further reason for adhering to the definitions of the genera Tubella and Trochospongilla as accepted by Potts and for considering that the species pennsylvanica should be retained in the genus Tubella. *Tubella paulula* (Bowerbank) 1863, Synonym *Spongilla paulula* Bowerbank 1863, is the type species of the genus Tubella, yet, in revising the genera according to Annandale's redefinition, Gee (1932) lists this species in the genus Trochospongilla. Now if the author correctly interprets the rules of nomenclature, the type species of a genus is the one fixed point of the genus and, so long as the genus stands, can not under any circumstances be transferred to a subsequently established genus. *Tubella paulula* must, therefore, remain in the genus Tubella. While the author has not had the opportunity of examining original materials, yet a comparison of her specimens of *Tubella pennsylvanica* with the descriptions and excellent figures of spicules of the various species of Tubella and Trochospongilla given by Gee (1931 and 1932) convinces her that the species pennsylvanica most closely resembles *Tubella paulula* in form of gemmule birotulates, and was, therefore, rightly placed in the same genus by Potts.

Spongilla Aspinosa

Since the presence or absence of microspines on the dermal spicules is the criterion which separates *Spongilla aspinosa* Potts 1880 from *Spongilla lacustris,* and since microspines may be lacking from the dermal spicules of *S. lacustris* grown in water lacking silica, the question naturally arises, Is *Spongilla aspinosa* a valid species or did Potts describe, as a distinct species, these silica poor forms of *S. lacustris?* To answer this question the author examined specimens of *S. aspinosa* collected by Potts from the type locality, Absecum, New Jersey, and kindly loaned for the study by the Academy of Natural Sciences of Philadelphia, and the United States National Museum. A microphotograph of this rare species, showing the end of one skeletal spicule and several dermal spicules, is given in Figure 26. A comparison of this figure with the silica poor *S. lacustris* (Fig. 24) shows at a glance that the robust, well defined, glassy looking dermal spicules of *S. aspinosa,* are distinct from the barely visible, incompletely silicified spicules of the silica deficient *S. lacustris.* So far as the author's work is concerned, *Spongilla aspinosa* Potts stands as a valid species.

SUMMARY

1. As the result of an examination of 127 lakes and 17 streams in northeastern Wisconsin 10 species of *Spongillidae* were discovered, of which one,

Ephydatia everetti, has not previously been reported for the state or the Great Lakes region. Six species were found in sufficient abundance to permit an ecological study of their habitats.

2. *Ephydatia mülleri* is usually found associated with *Spongilla fragilis* and *S. lacustris,* although differing from these two species in that it is less tolerant of waters extremely low in mineralization. It was never taken in waters having a bound CO_2 content below 5 mgms. per liter or a conductivity below 30 reciprocal megohms.

3. *Ephydatia everetti* and *Spongilla ingloviformis,* on the other hand, are restricted to waters low in mineral content, neither species having been found in waters having a bound CO_2 above 5 mgms. per liter or conductivity above 20 reciprocal megohms. The two species differ in that *E. everetti* is restricted also to waters low in SiO_2, color, and organic content, whereas *S. ingloviformis* appears indifferent to SiO_2 content and is favored by high color and high organic content.

4. *Spongilla lacustris,* although occurring throughout the entire range of conditions found, attains its best development in small lakes of high color and organic content and rather low mineralization.

5. The habitat selection of *Spongilla fragilis* is similar to that of *Ephydatia mülleri* except that it has a wider tolerance for practically all of the factors studied.

6. *Tubella pennsylvanica* was found widely distributed throughout the range of conditions studied, except that it was absent from the more basic and more highly mineralized waters. Like *Spongilla ingloviformis,* with which it is frequently collected, it is distinctly favored by bog conditions, high acidity, color and organic content.

7. Transparency of water is important in determining the position of sponges with respect to depth and shade, but not their presence or absence from lakes. *Ephydatia everetti* was collected at a depth of 3.5 meters in transparent water. No species, not even among those ordinarily restricted to shaded situations, was taken at a depth below the penetration of visible light rays.

8. Continuous water movement, not strong current, seems to favor *Ephydatia mülleri* and *Spongilla fragilis,* and to be necessary for a good growth of *Heteromyenia argyrosperma* or *Carterius tubisperma.* *Spongilla lacustris* shows better development in standing waters provided they are high in organic content, whereas *Tubella pennsylvanica* and *Spongilla ingloviformis* are practically restricted to such conditions.

9. Growth form of colonies is believed to be affected by age, current or wave action, area, smoothness and slope of the base of support, and amounts of organic and mineral solutes in the water.

10. Skeletal development is greatly influenced by the mineral content of

the water, especially SiO_2. In waters of SiO_2 content below 0.4 mgms. per liter and of low conductivity and total solutes, *Spongilla lacustris* shows a progressive attenuation of its spicules, eventually losing its microspined dermal spicules (an important species character). These skeleton-poor forms appear no less vigorous and thrifty than heavily-spiculed specimens from more highly mineralized waters. Similarly *Tubella pennsylvanica* shows marked variations correlated with the degree of mineralization of the water. These entirely normal variations, in some cases, abrogate accepted generic criteria.

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EXPLANATION OF PLATES

PLATE II—FIGS. 2-9

FIG. 2. *Ephydatia everetti* from Bass Lake, SiO_2, 0.2 mgms. per liter, showing skeletal spicules (acerates), gemmule spicules (large birotulates) and dermal spicule (small birotulate).

FIG. 3. *Ephydatia everetti* from Bear Lake, SiO_2, 0.8 mgms. per liter. Magnification the same as Fig. 1.

FIG. 4. *Ephydatia mülleri* from Little Rice Lake, showing a characteristic growth form.

FIG. 5. *Spongilla fragilis* from the Wisconsin River above a power dam. Observe irregular surface and protruding oscula.

FIGS. 6 and 7. *Spongilla fragilis,* dried specimens showing differences in skeletal structures. From the Wisconsin River and outlet of Trout Lake respectively.

FIGS. 8 and 9. *Spongilla igloviformis*, from Helmit Lake, showing large size and rugose surface developed under optimum conditions, also heavy external membrane and inconspicuous pores (Compare with *Spongilla fragilis*, Fig. 5).

PLATE II—FIGS. 10-17

FIGS. 10-16. Spicules of *Spongilla lacustris* from lakes of differing SiO_2, contents. Magnification the same in all figures.

FIG. 10 from North Twin Lake, SiO_2 content 13 mgms. per liter.

FIG. 11 from Papoose Lake, SiO_2 content 1.6 mgms. per liter.

FIG. 12 from Rock Lake, $Si0_2$ content 0.6 mgms. per liter.

FIG. 13 from Nebish Lake, SiO_2 content 0.4 mgms. per liter.

FIG. 14 *(lower left of plate)* from Mary Lake, SiO_2 content 0.25 mgms. per liter.

FIG. 15 *(center right of plate)* from Katinka Lake, SiO_2 content trace.

FIG. 16 from Joyce Lake, SiO_2 content trace.

FIG. 17. *Tubella pennsylvanica*, fragment of a gemmule, from a moderately soft water, Nellie Lake, showing inaequibirotulates with slender shafts, irregularly rayed proximal rotules, and knob shaped distal rotules.

PLATE III—FIGS. 18-26

FIGS. 18-23. Spicules of *Tubella pennsylvanica*. Magnification the same in all figures.

FIG. 18 from Little Rice Lake, SiO_2 content 7.0 mgms. per liter.

FIG. 19 from Perch Lake, SiO_2 content trace.

FIG. 20 from Little Rudolph Lake, SiO_2 content trace.

FIG. 21 part of a gemmule from Little Rudolph Lake, showing shape and sparse distribution of birotulate spicules characteristic of this species from very soft waters.

FIG. 22. Skeletal spicules of the same specimen.

FIG. 23. Gemmule and skeletal spicules from the same colony as figures 21 and 22, after 33 days of development in water of silica content 3.0 mgms. per liter. All gemmule spicules shown developed after the transfer. Skeletal spicules developed before and after transference to water of higher SiO_2 content are easily distinguished.

FIG. 24 and 25. *Spongilla lacustris* from Little Rudolph Lake.

FIG. 24. Three skeletal spicules and a group of dermal spicules from a colony collected from Little Rudolph Lake.

FIG. 25. Spicules from the same colony after 33 days of development in water of SiO_2 content 3.0 mgms. per liter. The two spicules crossing near the top of the picture developed in the water of higher SiO_2 content. The spicules near the bottom of the picture matured while still in Little Rudolph Lake.

FIG. 26. *Spongilla aspinosa* Potts. End of one skeletal spicule and several dermal spicules. This species is not to be confused with the forms of *Spongilla lacustris* which have non microspined dermal spuicules due to lack of silica in the water (Fig. 24).

PLATE I

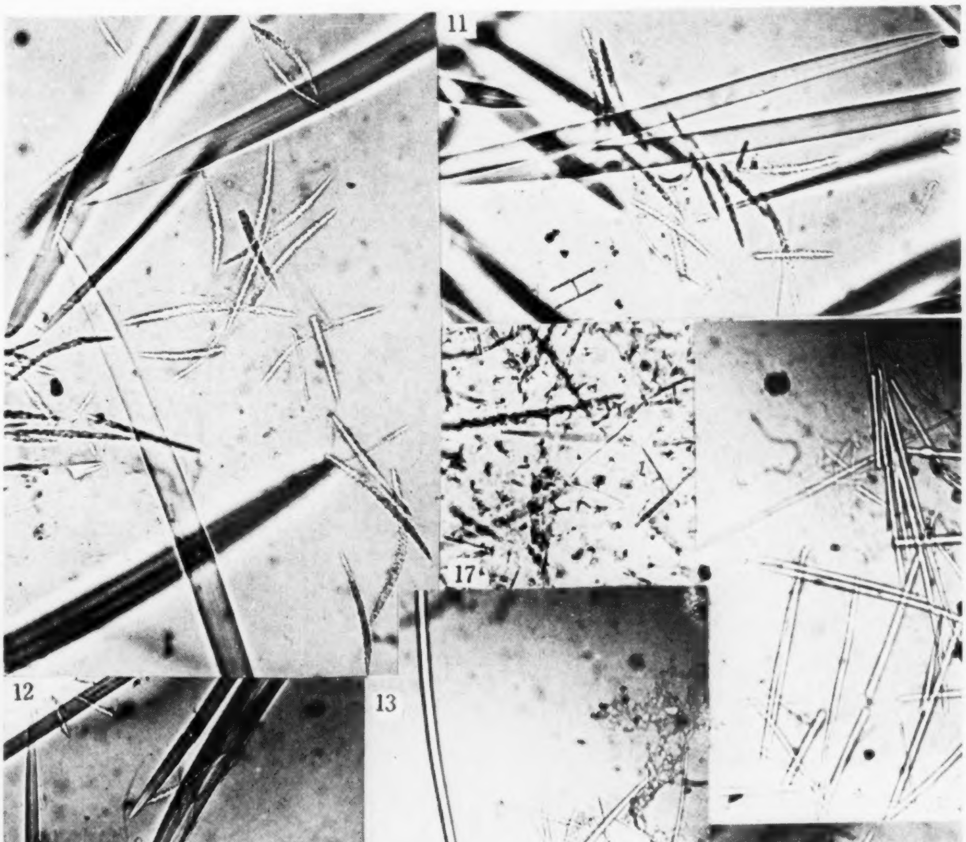

PLATE II

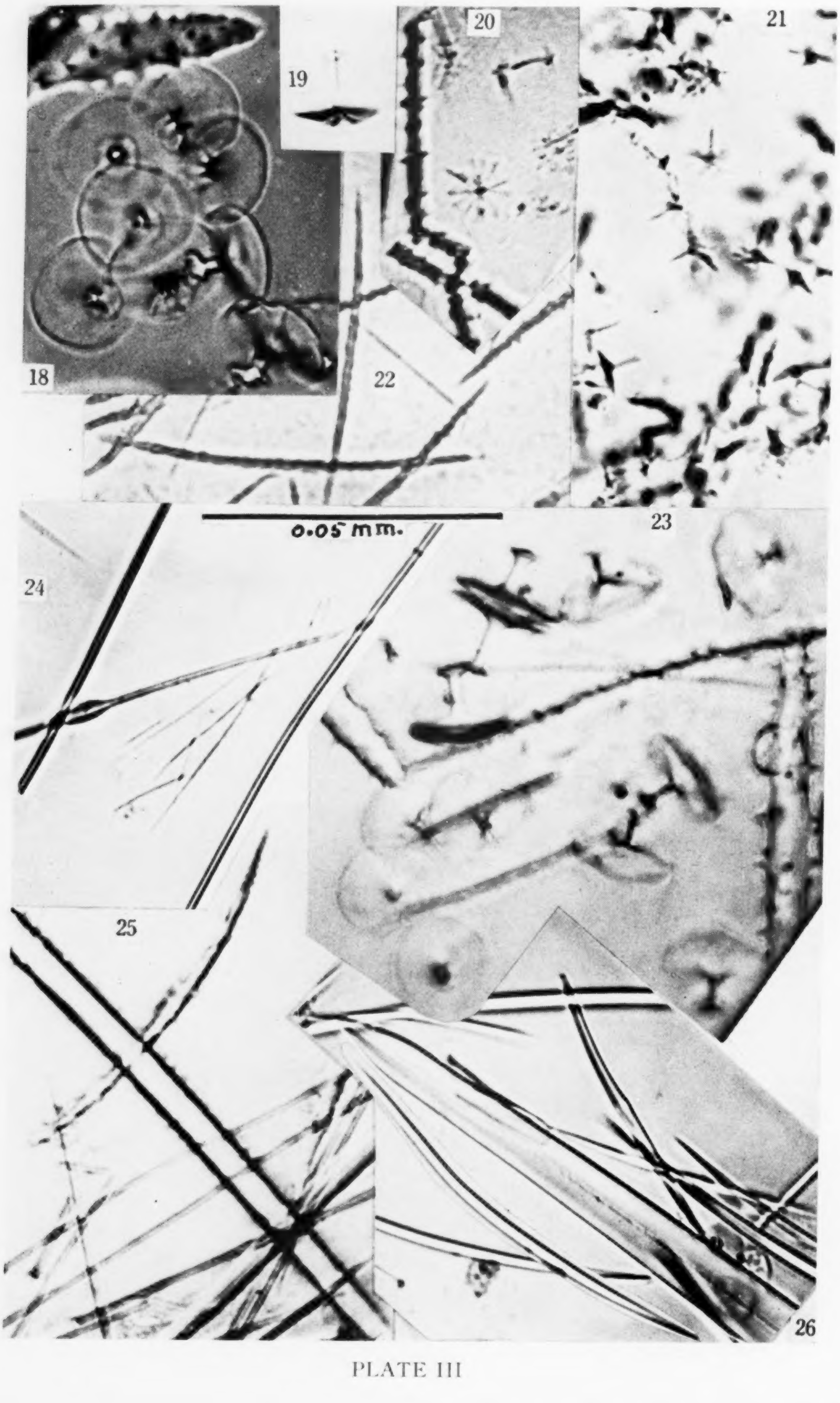

PLATE III

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